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Control of the Vetch Bruchid, *Bruchus brachialis*, in Oregon[1]

E. A. Dickason and J. D. Lattin, *Oregon State College, Corvallis*

Abstract

Insecticides were evaluated in Oregon for control of the vetch bruchid, *Bruchus brachialis* Fahr., in hairy vetch seed production in an attempt to reduce the possibility of a chemical residue on the small-grain companion crop normally seeded with vetch. The following toxicants were selected and their control performances compared with the standard DDT recommendation: methoxychlor, malathion, Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl)phosphorodithioate), and Dibrom® (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate) plus Volck® Supreme Oil.

All treatments reduced vetch bruchid populations, and seed collected from treated plots germinated in excess of 90%. In addition, the populations of the pea aphid (*Macrosiphum pisi* (Harris)) were reduced in those plots treated with an organic phosphate insecticide.

The vetch bruchid, *Bruchus brachialis* Fahr. (Coleoptera: Bruchidae), was first found in Oregon during 1936, and within 5 years it became a limiting factor in the production of hairy vetch seed, *Vicia villosa* Roth. Larval feeding within a seed impairs or completely destroys the viability of the seed. Seeds from which adults have emerged are considered inert matter in seed testing, but seed in which insect development is not complete vary in viability depending upon the tissue damaged by larvae and upon subsequent larval development (Pierpoint 1952).

Rockwood *et al.* (1945, 1946) conducted experiments under Western Oregon conditions and recommended a single application of 25 pounds of 3% DDT dust per acre for control of adults. The insecticide had no effect on hatching of eggs, and timing of treatment was based on the appearance of the first vetch pods and prior to egg deposition. During recent years, airplane spraying with 1 pound of actual DDT per acre has been the most common method of treatment. Poor control resulted when application was delayed until pods were present. Reinfestation of treated fields may occur following the cutting of nearby vetch hay fields or of escaped vetch growing along public road rights of way.

Vetch is normally seeded with one of the small grains (oats, wheat, barley, or rye) to support the weak vetch stems and reduce lodging (Henson & Schoth 1955). This practice presents a possible residue problem since DDT is not registered for use on small grains after heads begin to form (Anonymous 1960). Control experiments were conducted during 1960 to evaluate several insecticides other than DDT that would control the vetch bruchid and reduce the hazard of chemical residue on the small-grain companion crop.

Procedure.—A preliminary small-plot experiment was conducted to compare the effectiveness of various insecticides against the vetch bruchid. All insecticides evaluated appeared to be as effective as DDT in reducing adult populations for a period of 3 to 5 days, although there was some reinfestation of treated plots from the untreated area of the surrounding field. As a result, these insecticides and rates of application were repeated in a field experiment.

A 40-acre hairy vetch seed field was divided into five 8-acre plots. Each plot was treated by airplane (3 gallons of spray per acre) with one of the following insecticides: wettable powders, DDT, 50%; methoxychlor, 50%; emulsifiable concentrates, malathion, 56.8%; Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl) phosphorodithioate), 18.1%; Dibrom® (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate), 64.5%. The manufacturer suggested the addition of Volck® Supreme Oil (98% petroleum oil) to Dibrom. Insecticides were applied at the (approximate) rates per acre outlined in table 1.

An untreated plot was not included in the experimental field to avoid possible reinfestation of treated plots. Previous experiments with the vetch bruchid have established the necessity of this procedure. A nearby small field containing volunteer vetch was utilized as an untreated check plot. This field supported approximately the same bruchid population as the treated field (table 1), but had a much thinner stand of vetch.

Adult populations were determined by sweeping with a standard 15-inch insect net. Four samples, with 25 sweeps per sample, were collected from each plot before treatment and again 2 and 5 days after treatment. The material collected from each sample was placed in a plastic bag, refrigerated in the laboratory, and the insects were counted after floating off extraneous plant debris in a tray of detergent-water solution.

Five hundred vetch pods were collected from each plot immediately prior to dehiscence of the most mature pods. The pods on the lowest raceme were not sampled because application was unavoidably delayed 4 days and an occasional plant had produced lower pods before treatment. Each pod was examined and the number of vetch

[1] Approved for publication as technical paper number 1364, Oregon Agricultural Experiment Station. Accepted for publication December 9, 1960. Partial cost of publication of this paper was met by the Entomology Department, Oregon State College, Corvallis.

Table 1.—Effectiveness of insecticidal sprays applied by airplane to control the vetch bruchid in Oregon.

Insecticide and Ounces of Active Ingredient per Acre	Average Number Adults[a]			500-Pod Sample		Seeds from 500-Pod Sample			
	Before Treatment	2 Days After	5 Days After	Infested with Eggs (%)	No. Eggs per Infested Pod	Total	Infested with Larvae (%)	Producing Adults (%)	Germination[b] (%)
Untreated	53.5	36.2	54.9	99.2	9.6	1,618	76.7	64.5	20
DDT, 16	61.2	0.25	0.25	14.6	3.7	1,951	4.6	3.5	95
Methoxychlor, 16	59.2	0.75	none	8.4	3.6	1,815	1.9	1.4	97
Malathion, 16	46.0	none	0.25	15.6	2.1	1,849	3.5	3.0	93
Guthion, 8	29.7	0.25	0.25	9.8	4.7	1,651	4.8	2.4	93
Dibrom, 8[c]	39.2	none	1.0	9.4	1.2	1,923	1.4	0.8	98

[a] Population estimates based on average of four sample stations (25 sweeps per sample station) per plot.
[b] Including hard seeds.
[c] Plus 1 pint Volck Supreme Oil per acre.

bruchid eggs recorded. Seeds were removed after these pods had dried and the number of larval entrance holes in each seed recorded. The seeds were retained for subsequent adult emergence. Seed germination tests were conducted and evaluated by the Oregon State College Seed Testing Laboratory according to rules adopted by the Association of Official Seed Analysts. The germination data are based on the number of seeds which germinated plus the number of hard seeds in four 100-seed samples from each plot.

RESULTS AND DISCUSSION.—All the insecticides applied compared favorably with DDT in reducing vetch bruchid populations for a 5-day period following treatment (table 1). A later sampling for adults was not practical because the natural dropping of old florets from the newly formed pods added so much debris to the samples swept. Similarly, all insecticides compared favorably with the standard DDT recommendation in reducing the percentage of pods infested with eggs, number of eggs per infested pod, seed infestation, yield of adults from infested seeds and, in addition, they increased seed germination.

Timing of treatment was based on the appearance of the first vetch pods and prior to egg deposition. This delay was found necessary (Rockwood *et al.* 1945, 1946) to insure that all adults had left their overwintering quarters, but it results in an insecticide application to the crop when it is in full bloom. Hairy vetch is one of the leading honey producing plants, and pollination by honey bees and bumble bees is essential to good seed production (Scullen 1956). Johansen (1960) has classified these insecticides for field application as a spray as follows: Guthion, use Class I, hazardous to bees at any time; DDT and Dibrom, use Class II, not hazardous if applied when bees are not foraging; malathion, use Class II?; and methoxychlor, use Class III?, not hazardous to bees at any time.

Approximately 3 weeks after the application of the insecticides there was a striking difference in bloom between the treated and untreated vetch. This difference was apparent in both small-plot and large-plot experiments with all insecticides applied, as well as in areas in commercially treated fields where the airplane missed spraying DDT in order to avoid power lines or other tall objects. Untreated vetch maintained a heavy bloom, and by contrast treated vetch was almost free of bloom the fourth week following treatment. It appeared, but was not established experimentally, that the difference was in duration of bloom and not the result of excessive floret drop or failure of pods to set.

Although the experiment was designed to evaluate vetch bruchid control, the effect of the treatments on the pea aphid, *Macrosiphum pisi* (Harris), was evaluated. Populations of the pea aphid were low in hairy vetch fields during 1960. The results of counts given in table 2 are based on the average numbers of both adults and nymphs. The chlorinated hydrocarbon treatments (DDT and methoxychlor) had no apparent effect on aphid populations, but the organic phosphate treatments (malathion, Guthion, and Dibrom) all gave an initial suppression of aphid populations. The 5-day population given for malathion (table 2) is distorted because one of the samples used in computing the weighted average contained more than five times the number present in any of the other three samples. Unfortunately, all treatments killed the lady beetles that were present before treatment.

Table 2.—Effect of insecticides applied for vetch bruchid control on the population of the pea aphid.

Insecticide and Ounces of Active Ingredient per Acre	Average Number of Pea Aphids[a]		
	Before Treatment	2 Days After	5 Days After
Untreated	87.2	144	271.5
DDT, 16	35.2	69	172.5
Methoxychlor, 16	53.7	107	174.2
Malathion, 16	40	24.2	89
Guthion, 8	48	6.75	46
Dibrom, 8[b]	35.7	5.5	7.2

[a] Population estimates based on average of four sample stations (25 sweeps per sample station) per plot.
[b] Plus 1 pint Volck Supreme Oil per acre.

There was very little difference in the performance of the insecticides evaluated for vetch bruchid control (table 1). However, several factors must be considered in selecting an insecticide; these include: (1) the effect of treatment on pollinating insects, (2) the presence of aphids in fields (table 2), and (3) the registration of the insecticide on vetch, taking into account the specific small-grain companion crop usually involved.

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Parasites of the Jack-Pine Budworm, *Choristoneura pinus*, with Special Reference to Parasitism at Particular Stand Locations[1]

HERBERT M. KULMAN and ALEXANDER C. HODSON[2]

ABSTRACT

In Minnesota in 1958 seven of the 16 species of parasites that were reared from the jack-pine budworm, *Choristoneura pinus* Free., were not previously reported. Studies of parasites at various locations showed that parasitism by *Phaeogenes hariolus* (Cress.) and *Itoplectis conquisitor* (Say) was heavier on edge trees than on trees inside of stands. *I. conquisitor* parasitism was heaviest at the bottom of trees at the edge of stands and heavier on regressive trees (weak dominants and codominants) than on progressive trees (head dominants and strong dominants) inside stands. *Glypta fumiferanae* (Vier.) parasitism was heavier on trees inside stands than on edge trees and was heavier in the middle and top portion of the edge trees than on the bottom. On small plantation trees *G. fumiferanae* was more common on staminate-cone-producing trees.

In 1947 Baker recorded *Itoplectis conquisitor* (Say), *Phaeogenes hariolus* (Cress.), and unidentified tachinids as parasites of the jack-pine biological race of the spruce budworm. After the separate species designation for the jack-pine budworm by Freeman in 1953, three papers were published concerning the parasites of the jack-pine budworm. Parasites from Manitoba and Ontario were reported by Walley (1953) and parasites from Michigan by Benjamin and Drooz (1954) and Drooz & Benjamin (1956).

In the summer of 1958 collections were made of budworm pupae and pupae of *Glypta fumiferanae* (Vier.) and *Apanteles* sp. found on the sampled branches at three Minnesota locations. The pupal cases were kept in groups of about 25 in 2-inch diameter pasteboard shipping tubes with a fine mesh cloth cover. A few pupae were kept individually in small vials with a wad of tissue paper. The vials were stoppered with a cotton plug. A list of the parasites collected as well as the parasites reported by other investigators is given in table 1.

It is difficult to compare the findings because the Michigan studies were made on all life stages of the budworm, whereas the Manitoba and Ontario studies were made only on hymenopterous pupal parasites of the budworm and the Minnesota studies were made on all budworm pupal parasites and *Glypta fumiferanae* and *Apanteles* sp., larval parasites, whose pupal cases were present on the sampled branches. Of the 30 parasites listed in table 1, 18 were recorded in Michigan, 16 in Minnesota, 10 in Ontario, and 5 in Manitoba. At least 22 of these parasites have been reported also from the spruce budworm, *Choristoneura fumiferana* (Clem.).

The following seven parasites had not been previously reported from the jack-pine budworm:

Apanteles (undescribed species).

Apanteles polychrosidis Vier.

Mesochorus sp. (*M. diversicolor* Vier. has been reported as a hyperparasite in cocoons of primary parasites of the spruce budworm by Dowden *et. al.* (1948)).

Brachymeria compsilurae (Cwfd.), a hyperparasite reared from larvaevorids.

Tetrastichus coerulescens Ashm. which may or may not be a hyperparasite, Viereck *et al.* (1916). Twelve were reared from a single budworm pupa.

Madremyia saundersii (Will.).

Sarcophaga aldrichi Park. Because of the manner in which the material was stored an exact count of the dipterous parasites by species was not possible; however, more than 70 of the 325 dipterous larvae encountered were *S. aldrichi*.

From a group of pupae that were kept in individual containers it was found that *Amblymerus verditer* (Nort.) averaged 30 parasites per budworm pupal case and ranged from 16 to 44. The female-male ratio was 3 to 7. Counts were made from 18 parasitized pupae. From six pupae only males were reared and from two pupae only females were reared. In 16 of the 18 pupal cases 75% or more of the parasites were of one sex. In the remaining two pupal cases they were about evenly divided.

The collection procedure was designed to investigate the numbers of parasites at several stand locations.

[1] Paper No. 4436 Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul. Accepted for publication August 2, 1960.

[2] Assistant Professor of Entomology, West Virginia University and Professor of Entomology and Economic Zoology, University of Minnesota, respectively.

Table 1.—Parasites of the jack-pine budworm collected in Minnesota in 1958 compared with collections in Manitoba, Ontario and Michigan.[a]

Parasite	Collection Date and Location					Record on Spruce Budworm
	Minnesota 1958	Walley (1953) Manitoba 1951	Walley (1953) Ontario 1951	Benjamin & Drooz (1954) Michigan 1951-52-53	Drooz & Benjamin (1956) Michigan 1953	
		Hymenoptera				
Braconidae[b]						
Apanteles (undescribed species)	X	0	0	0	0	0
Apanteles fumiferanae Vier.	X	0	0	X	0	X
Apanteles polychrosidis Vier.	X	0	0	0	0	0
Meteorus trachynotus Vier.	0	0	X	0	0	X
Ichneumonidae[c]						
Apechthis ontario (Cress.)	0	X	X	X	X	X
Calliephialtes comstockii (Cress.)	0	0	X	0	0	X
Campoplex hyalinus (Prov.)	0	X	0	0	0	Z
Exochus sp.	0	0	0	X	0	Z
Gelis tenellus (Say)	0	0	X	0	X	X
Glypta fumiferanae (Vier.)	X	X	0	X	X	X
Itoplectis conquisitor (Say)	X	X	X	X	X	X
Mesochorus diversicolor Vier.[f]	Z	0	0	0	0	X
Phaeogenes hariolus (Cress.)	X	X	0	X	X	X
Scambus hispae (Harr.)[g]	X	0	X	X	X	X
Chalcidoidea[d]						
Amblymerus verditer (Nort.)[h]	X	0	0	X	X	X
Brachymeria compsilurae (Cwfd.)[i]	X	0	0	0	0	0
Brachymeria ovata (Say)	0	0	X	0	X	0
Eurytoma atripes Gahan	0	0	Y	0	0	0
Habrocytus phycidis Ashm.	0	0	X	Z	X	X
Spilochalcis sanguineiventris (Cress.)	0	0	Y	0	0	0
Syntomosphyrum esurus Riley	0	0	0	Y	X	0
Tetrastichus coerulescens Ashm.[j]	X	0	0	0	0	Z
Trichogramma minutum Riley	0	0	0	X	0	X
		Diptera[e]				
Larvaevoridae						
Actia interrupta Curr.	0	0	0	X	0	X
Aplomya caesar (Ald.)	X	0	0	0	X	X
Lypha setifacies (West.)	0	0	0	X	0	X
Madremyia saundersii (Will.)	X	0	0	0	0	X
Phryxe pecosensis Tns.	X	0	0	X	X	X
Sarcophagidae						
Agria affinis (Fall.)	X	0	0	X	X	X
Sarcophaga aldrichi Park.	X	0	0	0	0	X

[a] X, parasite recorded; Y, same genus, but only recorded as "near" the species; Z, same genus, but only recorded as "sp."; 0, no record.
[b], [c], [d], and [e] Identified by C. F. W. Muesebeck, L. M. Walkley, B. D. Burks, and C. W. Sabrosky, respectively, Entomology Research Division, U. S. Dept. Agric. (*S. aldrichi* identified by R. W. Smith, Canadian Dept. Agric.)
[f] Reported as a hyperparasite, Dowden *et al.* (1948).
[g] Reported as a primary or hyperparasite, Wilkes *et al.* (1948)
[h] Possibly a hyperparasite, Dowden *et al.* (1950).
[i] Hyperparasite of larvaevorids.
[j] Reported as a primary or hyperparasite. Viereck *et al.* (1916).

Collections of 100 or more pupae were made in the bottom middle, and top thirds of edge-of-stand trees and in the top third of progressive[2] and regressive[2] trees inside the stands. The following stands were sampled:

1. Jack pine plantation at Bemidji—27 years old, 5 inches DBH, and 37 feet tall
2. Jack pine plantation at Buena Vista—21 years old, 4 inches DBH, and 31 feet tall
3. Natural jack pine stand at Badoura—35 years old, 6 inches DBH, and 33 feet tall

Collections were also made from three 8- to 10-year-old and 8- to 12-foot-tall jack pine plantations near Badoura. Collections were made from six trees in each plantation. In addition to the systematic samples general collections of pupae were taken in each area.

The number of parasites collected at the various locations and stand positions are shown in table 2. On the larger trees (first three locations in table 2) it appears (1) that parasitism by *Glypta fumiferanae* is heavier on trees inside stands than on trees at the edge of stands, and that parasitism on edge-of-stand trees is heaviest in the upper portions of the crown, (2) that parasitism by *Itoplectis conquisitor* is heaviest at the bottom of trees at the edge of stands and is heavier on regressive trees than on progressive trees inside stands, and (3) that parasitism by *Phaeogenes hariolus* is heavier on trees at the edge of stands than on inside-of-stand trees. Jaynes

[2] As defined by Gevorkiantz *et al.* (1943): Progressive trees, head dominants and strong dominants; regressive trees, weak dominants and codominants.

Table 2.—Parasites reared from jack-pine budworm pupae and associated parasites[a] collected from jack-pine at several tree and stand positions at three Minnesota locations.

Location, Tree, Position, Grown Class	Per Cent								Total Pupae Collected
	Glypta fumiferanae	*Itoplectis conquisitor*	*Phaeogenes hariolus*	*Amblymerus verditer*	Other Hymenoptera	Diptera	Nonparasitized Inactive Pupae	Budworm Moths	
Bemidji[b]									
E bottom	0.7	24.4	4.4	0.0	0.7	14.8	34.8	20.0	135
E middle	17.8	9.1	1.5	0.5	1.0	9.1	46.2	14.7	197
E top	19.7	8.6	3.9	2.6	0.7	4.6	38.2	21.7	152
R top	23.2	10.3	1.3	0.6	2.6	7.1	26.4	28.4	155
P top	21.2	5.9	1.8	1.2	0.6	6.5	42.9	20.0	170
Total	17.1	11.1	2.5	1.0	1.1	8.3	38.3	20.6	809
Buena Vista[b]									
E bottom	1.2	6.7	3.0	1.8	0.6	7.9	29.3	49.4	164
E middle	6.1	1.4	1.4	3.4	0.7	4.1	25.0	53.4	148
E top	5.4	3.4	0.7	3.4	1.4	6.1	34.7	44.9	147
R top	8.0	3.6	0.0	2.2	0.7	8.0	31.9	45.6	138
P top	7.4	2.5	0.0	0.8	0.8	3.3	49.2	36.1	122
Total	5.4	3.6	1.1	2.4	0.8	6.0	34.3	46.3	719
Badoura[b]									
E bottom	6.5	2.9	4.3	2.2	0.7	10.8	15.1	57.5	139
E middle	10.7	0.0	0.8	0.0	0.8	8.4	22.9	56.5	131
E top	8.0	0.7	2.0	0.0	0.0	23.5	19.4	46.2	149
R top	12.1	2.0	0.0	2.0	0.0	11.1	22.2	50.5	99
P top	15.6	1.4	0.7	0.7	0.7	17.7	18.4	44.9	147
Total	10.5	1.4	1.7	0.9	0.5	14.7	19.4	50.9	665
Plantations at Badoura[c]									
Plantation 1									
Edge trees	3.4	3.9	2.2	5.6	0.0	20.2	20.2	44.4	178
Inside trees	7.1	4.8	1.2	3.6	0.0	13.1	18.4	51.8	168
Cones, ♂	6.8	3.4	2.6	2.6	0.0	12.8	18.8	52.9	234
Cones, no ♂	1.8	6.3	0.0	8.9	0.0	25.0	20.5	37.5	112
Plantation 2									
Edge trees	3.5	4.4	0.0	2.2	0.4	13.1	25.7	50.9	230
Inside trees	2.9	2.6	0.7	3.3	0.0	19.9	25.8	45.0	307
Cones, ♂	4.0	2.8	0.2	3.1	0.2	19.0	27.0	43.5	425
Cones, no ♂	0.0	5.4	0.9	1.8	0.0	8.9	20.5	62.5	112
Plantation 3									
Edge trees	0.0	3.0	0.8	1.5	0.0	10.6	22.7	61.4	132
Inside trees	3.6	0.0	1.8	5.4	0.0	10.7	35.7	42.9	56
Cones, ♂	1.5	2.2	1.5	3.0	0.0	8.2	24.6	58.9	134
Cones, no ♂	0.0	1.9	0.0	1.9	0.0	16.7	31.5	48.1	54

[a] *Glypta fumiferanae* and *Apanteles* sp. pupae were found on the branches from which the budworm pupae were collected.
[b] E, edge of stand trees; R, regressive trees; and P, progressive trees.
[c] Trees were 8 to 12 feet tall and at least 300 feet from larger stands.

(1954) found a heavier occurrence of parasitism of overwintering budworm larvae by *Glypta fumiferanae* and *Apanteles fumiferanae* Vier. in the upper crowns of balsam fir.

There appeared to be a positive correlation between the presence of staminate cones and parasitism by *Glypta fumiferanae* in the small plantations at Badoura (last three locations in table 2).

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Functions of the Madeira Cockroach (*Leucophaea maderae*) Alimentary Tract in the Absorption, Metabolism, and Excretion of Ronnel[1]

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ABSTRACT

The role of the alimentary tract of the Madeira cockroach, *Leucophaea maderae* (F.), in the *in vivo* processes of absorption, transport, metabolism, and excretion of P^{32}-labeled ronnel (*O,O*-dimethyl *O*-(2,4,5-trichlorophenyl) phosphorothioate) was investigated together with the qualitative and quantitative nature of the phosphorus-containing metabolites eliminated.

The wall of the foregut was found to be permeable to orally injected ronnel but not to certain of its water soluble hydrolysis products while the hindgut was permeable to both. The midgut primarily accumulated the water soluble metabolites when direct movement of ronnel from the foregut was blocked, and evidence suggests it as a site for hydrolytic degradation of the insecticide.

More than 97% of the radioactivity accumulating in the hindgut was in the form of water-soluble degradation products with about two-thirds entering with the discharge of the malpighian tubules. Only small quantities of radioactivity passed directly from the mid- to the hindgut.

Sublethal doses of ronnel progressively inhibited elimination of excreta while the hydrolysis products had little effect.

The major metabolite of ronnel eliminated in the feces was identified as dimethyl thiophosphate. This compound gradually diminished with time while the minor metabolites dimethyl phosphate and monomethyl ronnel increased.

The role of the insect alimentary tract in determining the fate of an ingested or absorbed insecticide is not well known. Roan *et al.* (1950) discovered that the foreguts of American cockroaches, *Periplaneta americana* (L.), selectively accumulated radioactive material when the insects were treated topically with TEPP. Similar results were later reported with parathion (Ball & Beck 1951, Fernando *et al.* 1951), pyrethrins (Zeid *et al.* 1953), DDT (Robbins & Dahm 1955), Bayer L-13/59 (dimethyl 1-hydroxy-2,2,2-trichloroethyl-phosphonate) and DDVP (Acree *et al.* 1956), and the thiono isomer of demeton (March *et al.* 1955). These observations imply foregut permeability to certain organosoluble molecules circulating in the hemolymph but other routes of entry are possible such as the cardiac valve as suggested by Acree *et al.* (1956), and Jochum (1956) proposed secretion with the saliva into the oral cavity and ingestion with the food. There is also the question as to whether the radioactivity penetrating the foregut was the insecticide or its more water soluble metabolites.

When parathion, para-oxon, or TEPP was fed to *P. americana* there was a progressive decrease in the crop concentration and an increase in the mid- and hindgut with time. TEPP, the most water soluble compound of the three, diffused from the crop at the slowest rate (Fernando *et al.* 1951). March *et al.* (1955) have found a similar situation occurring in *P. americana* treated topically or orally with demeton, but they were also able to measure an increase in water-soluble demeton metabolites in the mid- and hind-gut with time.

Little is known concerning the interactions of insecticides and the midgut except that it is generally regarded as the site of absorption and possible lethal action of the arsenical insecticides (Forgash 1959). *In vitro* oxidation of parathion (Metcalf & March 1953) and malathion (O'Brien 1957) to their respective phosphates by the midgut tissues has been reported, but the foregut and malpighian tubule preparations were more active in catalyzing the oxidation of parathion.

Louloudes (1958) treated Madeira cockroaches topically with P^{32}-ronnel and observed that the hindgut accumulated greater quantities of labeled phosphate, all of which was water soluble, than the other gut regions.

The present investigation was concerned with elucidating the *in vivo* absorption, transport, metabolism, and excretion of orally administered ronnel (*O,O*-dimethyl

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O-(2,4,5-trichlorophenyl) phosphorothioate) in the Madeira cockroach, *Leucophaea maderae* (F.). Ronnel was selected for study because of its oral as well as contact toxicity to insects and its demonstrated ability when administered orally to the mammal host to kill blood-sucking flies and mosquitoes (Hewitt *et al.* 1958, Drummond 1958).

MATERIALS AND METHODS.—Phosphorus^{32}-labeled ronnel was prepared essentially by the methods of Louloudes (1958). Phosphorus trichloride (PCl_3) sealed in quartz ampoules was submitted for A.E.C. service irradiation at a flux of 5×10^{11} neutrons per square cm. per second for 3 weeks. Sulfur^{35}-labeled impurities were eliminated by fractional distillation of the PCl_3 from pure n-decane through a 12-inch column packed with $\frac{1}{8}$-inch glass helices. Radioisotope purity was verified by comparing the per cent absorption of the $P^{32}Cl_3$ and a $H_3P^{32}O_4$ standard by an aluminum disk (39.66 mg./cm.^{2}).

Thiophosphoryl chloride ($PSCl_3$) was synthesized by adding PCl_3 slowly to an equal molar amount of sulfur dissolved in dry carbon disulfide solvent in the presence of an aluminum chloride catalyst. The mixture was stirred during addition and the $PSCl_3$ was purified by distillation (b.p. 121° to 124° C.). Dry n-pentane was added to the product and the flask fitted with reflux condenser and a dropping funnel containing freshly prepared sodium methoxide (2:1 molar ratio) in dry methanol. The contents of the flask were cooled in a dry ice-acetone bath and the methoxide added dropwise with stirring. After warming to room temperature, water was added to the reaction mixture and the dimethyl phosphorochloridothionate, $(CH_3O)_2PSCl$, extracted with n-pentane by replacement of the reflux condenser with a liquid-liquid extractor. The extracts were dried with anhydrous sodium sulfate and transferred to a vacuum distillation apparatus. The solvent was removed under reduced pressure followed by $(CH_3O)_2PSCl$ which distilled at 36° to 40° C./1.5–2.0 mm. Hg.

Ronnel was prepared by adding $(CH_3O)_2PSCl$ dropwise to potassium 2,4,5-trichlorophenate in dry acetone solvent followed by gentle refluxing for one hour. The acetone was removed under vacuum and the residue in the flask partitioned between purified Skellysolve B and water. The Skellysolve layer was then washed once with 5% potassium carbonate and twice with water. The solvent layer was dried with anhydrous calcium chloride, concentrated, and the product crystallized by cooling. The labeled ronnel was further purified by recrystallization from dry methanol (m.p. 39° to 41° C.) and was found to be radiochemically pure (>99%) when analyzed by normal and reverse phase paper chromatography. The observable specific activity was 1×10^3 c.p.m./μg. for each synthesis.

Paper chromatographic analysis of the water layer of the final reaction mixture revealed that 80% of the radioactivity behaved as monomethyl ronnel (*O*-methyl *O*-hydrogen *O*-(2,4,5-trichlorophenyl) phosphorothioate) and 20% as dimethyl thiophosphate. The water was removed by lyophilization and the hydrolysis products purified for study.

Two one-dimensional chromatographic systems have been used successfully for separating ronnel, its oxygen analog *O,O*-dimethyl *O*-(2,4,5-trichlorophenyl) phosphate, and the various hydrolysis products (table 7).

The normal phase method consists of 85 parts purified Skellysolve B saturated with a mixture of 10 parts methanol and 5 parts acetic acid (v/v). The excess methanol-acetic acid is separated and the mobile phase transferred to suitable chambers lined with Whatman No. 1 paper. One-inch strips of Whatman No. 1 filter paper are coated with a 6% solution of Carbowax 4000 in chloroform (w/v). The reverse-phase method has a mobile phase consisting of 56 parts water, 42 parts absolute ethanol, and two parts chloroform (v/v). Whatman No. 1 strips, coated with a 5% solution (v/v) of Dow Corning Silicone 550 in acetone, are developed in unlined chambers.

An additional system used to separate some of the possible degradation products of ronnel consisted of a mobile phase of 65 parts isopropanol, 25 parts concentrated ammonium hydroxide, and 10 parts pyridine (v/v). Uncoated Whatman No. 1 paper strips were developed in unlined chambers (table 8).

Ronnel and its oxygen analog were detected by spraying the filter paper strips with an ammoniacal silver nitrate reagent (Price & Dietrich 1956) and the ionic phosphorus compounds with the ammonium molybdate reagent of Hanes & Isherwood (1949). Additional reagents used to detect ronnel and its sulfur-containing intermediates were acid permanganate or 2,6-dibromo-*N*-chloro-*p*-quinoneimine (Menn *et al.* 1957).

Qualitative analysis of the labeled ronnel and its metabolites separated on paper chromatograms was accomplished with a gas flow GM counter, chromatogram scanner, and synchronous chart drive recorder. Peaks on the chart representing radioactive zones were compared with the color zones of added nonlabeled standards. A relative percentage estimate of each labeled metabolite present in the extracts was obtained by measuring the area of each peak with a planimeter.

Cleanup procedures for paper chromatographic analysis of the chloroform extracted radioactivity were similar to those of Jones & Riddick (1952). Water-soluble radioactivity in the tissue homogenates and feces was concentrated when necessary by lyophilization.

Adult virgin female Madeira cockroaches from 2 to 4 weeks of age and from 1.8 to 2.2 grams in weight were used as experimental animals. The nymphs were reared and maintained under uniform conditions at 25° to 28° C.

The labeled compounds dissolved in propylene glycol were administered with a curved, blunt, No. 27-gauge needle attached to $\frac{1}{4}$ cc. hypodermic syringe mounted on a micrometer-driven microdrop applicator. The desired dose was thus injected quantitatively into either the foregut or hindgut via their external openings in a total volume of five μl. The mean doses calibrated by radioassay of drops from the syringe were 50.55 μg. of P^{32}-ronnel per cockroach (25 μg./gm.) and 139.25 μg. per cockroach (70 μg./gm.) for the hydrolysis products. In the case of the hindgut injections, the anus was sealed with paraffin to prevent elimination of the compound during the 24-hour period that the cockroaches were held.

Holding containers were one-pint, wide-mouth jars inverted over petri dish covers for the collection of fecal pellets. A $\frac{1}{4}$-inch mesh hardware cloth disk formed the

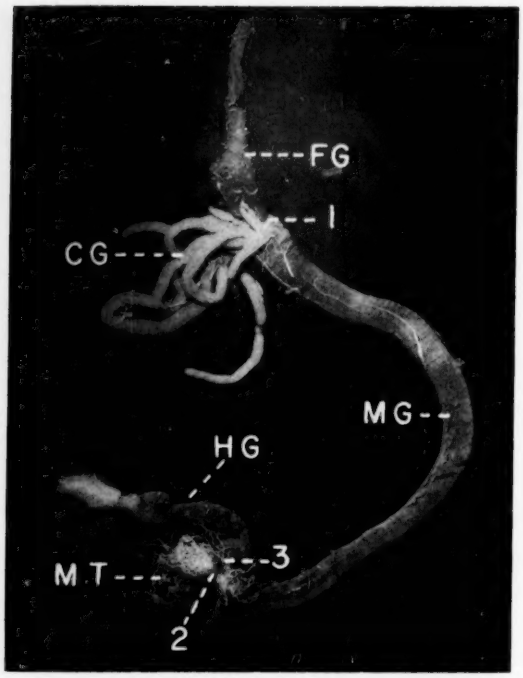

FIG. 1.—The alimentary tract of the Madeira cockroach, *Leucophaea maderae* (F.).

1. Ligature site between foregut and midgut.
2. Ligature site anterior to malpighian tubules.
3. Ligature site posterior to malpighian tubules.

CG. Caeca, gastric; FG, Foregut; HG, Hindgut; MG, Midgut; MT, Malpighian tubules.

floor of the cage while a food pellet and cotton-plugged water vial were wired in vertical position above the floor.

The gut was ligated at one of the following sites according to the experiment: between the foregut and midgut, immediately anterior to the base of the malpighian tubules, or immediately posterior to the tubules (Fig. 1).

Ligation was performed on cockroaches completely anesthetized with CO_2 to prevent muscle movement and loss of hemolymph. Two parallel longitudinal incisions were made across two tergites lateral to the dorsal vessel, the location depending on the site of the ligature. A third cut through the intersegmental membrane between the incisions formed a flap of integument which was lifted forward to expose the dorsal trachea and muscles. An opening was made through the dorsal diaphragm and the digestive tract ligated with waxed cotton thread. The tissues were kept moist during the operation by applying physiological saline (Yeager 1939) to the exposed parts. The integument was closed and the incision sealed with a thin layer of paraffin. The insects were then dosed while still anesthetized and placed in holding containers. Ligated control cockroaches survived for periods of 1 week or longer although the experimental time never exceeded 24 hours. A trypan blue solution injected into the ligatured foregut demonstrated complete blockage of the gut at the site of ligation.

Prior to dissection the insects were anesthetized with CO_2, wrapped in aluminum foil and quick-frozen with dry ice. The cockroaches were then thawed and the digestive tract, freed as completely as possible of extraneous tissue and malpighian tubules, was removed *in toto* together with the head and again frozen over dry ice in a petri dish. The gut was sectioned while frozen solid into its three main divisions, foregut, midgut and gastric caeca, and hindgut, while the body of cockroach minus wings and head was designated as "remainder." The tissues and their contents in the case of the gut were rapidly weighed on aluminum foil and kept frozen in covered petri dishes until assays could be performed.

Total recoveries of the administered doses in the insect were not obtained because of losses caused by defecation and possibly regurgitation of material containing radioactivity.

Total quantities of labeled ronnel and its metabolites were assayed by counting aliquots of tissue homogenates prepared in pyrex tissue grinders and adjusted to known volumes in glass-stoppered centrifuge tubes. Samples were prepared by the methods of Robbins *et al.* (1956). Phosphorus^{32}-labeled organosoluble compounds were extracted from the homogenates with equal volumes of chloroform, the solvent separated by centrifugation, and assayed by the preceding techniques.

To determine the efficiency of the extracting procedures P^{32}-ronnel was added to control homogenates of cockroach tissue, thoroughly mixed, and homogenate samples assayed for total radioactivity. Extraction with an equal volume of chloroform resulted in 99.3% mean recovery of the ronnel.

Samples were prepared in duplicate and sufficient counts accumulated with a gas flow GM counting system to attain a maximum standard error of 5%. Mean counting rates were then corrected for background, decay, and self absorption where necessary and were converted to microgram equivalents of the parent compound.

RESULTS AND DISCUSSION.—*Role of the Foregut.*—Permeability of the foregut wall to ronnel and certain of its hydrolysis products was investigated by preventing direct transport of the injected compound to the midgut with a ligature between the proventriculus and cardiac valve. From the data in table 1 it is apparent that ronnel readily penetrates the foregut wall as the hindgut and "remainder" tissues of the ligated cockroaches contained nearly the same quantities of radioactivity as the normal insects. From the percentage of the total dose remaining in the crop after 24 hours, nearly 25% entered the body of the ligated cockroach by transport through the foregut wall. Since 50% of the total dose disappeared from the foreguts of normal cockroaches, at least 25% was estimated to have passed through the cardiac valve directly into the midgut.

Eisner (1955) demonstrated the permeability of the foregut to fats by observing the accumulation of lipoid droplets in the epithelium of the crop wall of *P. americana* after feeding. *In vivo* experiments by Treherne (1958) indicate that although the foregut may be permeable to fats nearly all of the absorption probably takes place in

Table 1.—The distribution of labeled compounds in normal and ligated Madeira cockroaches 24 hours after oral injection of P^{32}-ronnel.[a]

Tissue		Total μg.		μg./Gm. Tissue		Per Cent Total Dose	
		A	B	A	B	A	B
Foregut	H	25.16	37.30	250.83	349.04	49.8	73.8
	C	19.50	34.03	195.20	312.60	38.6	67.3
	M	22.5	8.8	—	—	11.2	6.5
Midgut	H	3.14	1.41	33.38	9.12	6.2	2.8
	C	1.13	0.15	11.61	0.96	2.2	0.3
	M	64.0	89.4	—	—	4.0	2.5
Hindgut	H	1.97	1.87	40.32	30.61	3.9	3.7
	C	0.06	0.05	1.27	0.87	0.1	0.1
	M	97.0	97.3	—	—	3.8	3.6
Remainder	H	8.26	6.98	5.73	4.78	16.3	13.8
	C	2.60	3.60	1.78	2.45	5.1	7.1
	M	68.5	48.4	—	—	11.2	6.7

A = Normal cockroaches. B = Cockroaches ligated between foregut and midgut. H = Total homogenate. C = Chloroform soluble fraction. M = Per cent metabolized.

[a] Data expressed as μg.-equivalents of ronnel per cockroach are the mean of three replicates of three cockroaches each.

Table 3.—The absorption and translocation of P^{32}-hydrolysis products[a] of ronnel from the foregut of normal and ligated Madeira cockroaches 24 hours after oral treatment.[b]

Tissue	Operation	Total (μg.)	μg./Gm. Tissue	Per Cent Total Dose
Foregut	A	36.30	533.18	25.6
	B	84.42	547.24	60.6
Midgut	A	40.09	317.08	28.8
	B	0.04	0.30	—[c]
Hindgut	A	6.27	144.54	4.5
	B	0.05	1.10	—[c]
Remainder	A	19.36	14.03	13.9
	B	2.80	2.08	2.0

A = Normal. B = Ligated between foregut and midgut.

[a] 80% monomethyl ronnel and 20% dimethyl thiophosphate.

[b] Data expressed as μg.-equivalents of administered compounds per cockroach are the mean of two replicates of three cockroaches each.

[c] Less than 0.1% of total dose.

the gastric caeca and anterior ventriculus of the cockroach. Because of the permeability of the foregut wall to ronnel and its slow loss from that organ, it is believed that a significant quantity of the insecticide does enter the hemolymph through the crop epithelium.

Isolating the foregut from the midgut decreased the quantity of insecticide converted to water soluble metabolites in the crop. This suggests that hydrolytic enzymes from the midgut may be responsible for the degradation occurring in the foregut or that water-soluble compounds accumulating in the midgut may flow forward with the digestive secretions, thereby increasing the quantity of ionic metabolites in the foregut contents.

Ligatures placed between the midgut and hindgut also inhibited movement of insecticide from the foregut to the midgut, particularly when the blockage occurred anterior to the openings of the malpighian tubules (table 2).

The foregut appeared to be nearly nonpermeable to the hydrolysis products of ronnel (table 3). Quantitatively only 2% of the total dose was recovered in other tissues when the metabolites were injected into the ligated foregut, whereas in normal insects 47% was found.

Table 2.—The distribution of labeled compounds in ligated Madeira cockroaches 24 hours after oral injection of P^{32}-ronnel.[a]

Tissue		Total (μg.)		μg./Gm. Tissue		Per Cent Total Dose	
		A	B	A	B	A	B
Foregut	H	42.93	32.45	357.29	240.16	84.9	64.2
	C	36.01	27.40	297.71	205.94	71.2	54.2
	M	16.1	15.6	—	—	13.7	10.0
Midgut	H	1.86	3.16	13.02	26.41	3.7	6.2
	C	0.75	0.35	4.80	2.99	1.5	0.7
	M	59.7	88.9	—	—	2.2	5.5
Hindgut	H	1.56	0.50	26.55	13.64	3.1	1.0
	C	0.04	0.06	0.61	1.24	0.1	0.1
	M	97.4	88.0	—	—	3.0	0.9
Remainder	H	4.71	8.18	3.40	5.64	9.3	16.2
	C	1.51	3.07	1.08	2.12	3.0	6.1
	M	67.9	62.5	—	—	6.3	10.1

A = Gut ligated anterior to openings of malpighian tubules. B = Gut ligated posterior to openings of malpighian tubules. H = Total homogenate. C = Chloroform soluble fraction. M = Per cent metabolized.

[a] Data expressed as μg.-equivalents of ronnel per cockroach are the mean of three replicates of three cockroaches each.

Role of the Midgut.—It has been demonstrated that the midgut directly received at least half of the insecticide leaving the foregut in 24 hours. When this source of translocation was blocked by a ligature, the quantity of radioactivity in the midgut dropped to less than half of that in the normal cockroaches, as might be expected (table 1). The radioactivity which entered the midgut from the surrounding body tissues was nearly 90% in the form of water-soluble phosphates. This suggests that the midgut may selectively accumulate the polar metabolites or that ronnel is rapidly degraded during passage through the midgut epithelium. Evidence indicates that both processes may occur.

Isolation of the midgut from the foregut resulted in a large decrease of water soluble metabolites in both the foregut and "remainder" tissue, suggesting its importance as a site of degradation.

Several workers have reported the selective accumulation of phosphates by the midgut. Lindsay & Craig (1942) observed a concentration of phosphorus[32] in the midgut epithelium but very little in the foregut or hindgut epithelium, when insects were fed $NaH_2P^{32}O_4$. Hoskins & Harrison (1934) reported relatively high levels of inorganic phosphate in the midgut and hemolymph of honey bees and postulated a cycle in which inorganic phosphates are secreted into the midgut to take part in the buffering of digestive juices and are later reabsorbed by the hindgut. In the silkworm, *Bombyx mori* (L.), the midgut incorporates orally ingested $H_3P^{32}O_4$ into nucleic acids, phospholipids, acid soluble phosphates and phosphoproteins. Acid soluble phosphates increase rapidly just before cocoon spinning, suggesting the secretion of phosphates into the midgut cells from other parts of the body at this time. The posterior midgut was stated to be the region of the gut actively absorbing phosphorus because it was found to contain the highest levels of radioactivity (Ito *et al.* 1958).

Role of the Hindgut.—The quantities of labeled com-

Table 4.—Absorption of P^{32}-ronnel from the ligated hindgut of Madeira cockroaches 24 hours after rectal injection.[a]

TISSUE	TOTAL (μG.)	μG./GM. TISSUE	PER CENT TOTAL DOSE
Foregut	3.30	17.73	6.5
Midgut	15.26	113.53	30.2
Hindgut	9.66	213.04	19.1
Remainder	25.01	19.38	49.5

[a] Data expressed as μg.-equivalents of ronnel per cockroach are the mean of two replicates of three cockroaches each.

Table 5.—Absorption of P^{32}-hydrolysis products[a] of ronnel from the ligated hindgut of Madeira cockroaches 24 hours after rectal injection.[b]

TISSUE	TOTAL (μG.)	μG./GM. TISSUE	PER CENT TOTAL DOSE
Foregut	4.50	39.12	3.2
Midgut	15.12	76.10	10.9
Hindgut	49.55	901.88	35.6
Remainder	26.93	20.54	19.3

[a] 80% monomethyl ronnel and 20% dimethyl thiophosphate.
[b] Data expressed as μg.-equivalents of administered compounds per cockroach are the mean of two replicates of three cockroaches each.

pounds found in the hindgut were approximately the same in both ligatured and normal cockroaches except where the blockage occurred posterior to the openings of the malpighian tubules (table 2). Here the amount of radioactivity was less than one-third of that in the other experiments. This demonstrated that the source of an appreciable amount of hindgut radioactivity was the excretion of water-soluble metabolites by the malpighian tubules into that organ; the remainder, or about one third of the total radioactivity, being absorbed directly through the wall of the hindgut.

When the ligature was placed anterior to the openings of the malpighian tubules, the amount of radioactivity in the hindgut was diminished only slightly, indicating that minor quantities of the insecticide or its metabolites were passed directly from the mid- to the hindgut (table 2). Less than 3% of the radioactivity in the hindgut was in the form of unchanged ronnel except where the excretory products of the malpighian tubules were prevented from entering the hindgut. In this instance the amount of water soluble metabolites increased by approximately 30% in the midgut from the discharge of the tubules and dropped from 97.4% to 88.0% in the hindgut.

Permeability of the hindgut was investigated by injecting the labeled compounds directly into the lumen of the organ ligated posteriorly to the malpighian tubules followed by assay of the tissues 24 hours after treatment. P^{32}-ronnel readily penetrated the wall of the hindgut with only 19% of the total dose remaining in that organ after 24 hours, suggesting that the hindgut is more permeable to the insecticide than the foregut (table 4). This is consistent with the view that the hindgut functions as a reabsorption site for water, ions, and food material in the feces (Waterhouse & Day 1953, Ramsay 1958).

The hindgut was also readily permeable to the hydrolysis products but less so than to ronnel (table 5). The almost complete impermeability of the foregut to the water-soluble metabolites of ronnel, contrasted to that of the hindgut, is striking and *in vitro* observations substantiating this have been recorded. Dilute aqueous solutions of HCl and KOH were found to penetrate the wall of the isolated hindgut much faster than the wall of the foregut of *Blatta orientalis* L. That the crop was far less permeable to these materials than the hindgut was hypothesized to be caused by the greater thickness of the foregut cuticular intima (Eidmann 1922). Abbott (1926) also demonstrated that water and water-soluble substances, such as glucose and methylene blue, did not penetrate the wall of the isolated crop of the Australian cockroach, *Periplaneta australasiae* (F.).

Patton & Craig (1939) observed that the rectal region of the hindgut of the mealworm was most active in the reabsorption of electrolytes and nitrogenous compounds. A ligature placed immediately in front of the rectum greatly reduced absorption from the hindgut.

Malpighian Tubules.—The labeled phosphate moieties discharged in the urine of the malpighian tubules appear to be primarily the water-soluble metabolites of ronnel as described previously. The tubules either selectively absorb only the ionic metabolites or hydrolysis of the parent compound occurs during transport across the tube wall.

The uptake of electrolytes and nitrogenous compounds by the malpighian tubules of the mealworm has been studied by Patton & Craig (1939). Their findings indicated there was a nonselective absorption of Na, K, Ca, and Cl ions and glycine, glutamic acid, uric acid, and urea. Larger molecules such as gelatin were excluded from the tube filtrate. Ramsay (1956) has observed an exception in that potassium ions were actively secreted into the tube lumen of the stick insect, *Dixippus morosus* (Brunner), while other ions simply diffused in. He also reported that the phosphate concentration in the urine was greater than in the medium of deproteinized hemolymph and Ringer solution from which it was absorbed by isolated tubules. The rate of urine flow from the tube was found to increase with an increase of phosphate in the serum medium. From these observations one might speculate that the ionic phosphate metabolites of ronnel are selectively filtered from the hemolymph by the malpighian tubules as was evidenced by the large accumulation in the gut of water soluble radioactivity from this source. As yet the tube permeability to organosoluble compounds has not been investigated. The other possibility of ronnel metabolism or further degradation of metabolites in the tubules very likely occurs to some degree.

Excretion.—The effect of oral doses of ronnel and certain hydrolysis products on weight of excreta produced and the rate of excretion of labeled degradation products is summarized in table 6. The weight of feces eliminated by ronnel-treated cockroaches was greatly diminished from 48 to 96 hours following treatment while the water-soluble derivatives appeared to have little effect. Cockroaches displaying symptoms of intoxication produced fluid excreta by 72 hours. No mortality resulted from this level of exposure to the insecticides so these data suggest that ronnel has a direct adverse effect on the processes of waste elimination.

The peak of metabolite excretion following both treatments was reached at the 48-hour posttreatment interval. Although the ronnel-treated cockroaches voided less feces, they eliminated slightly more of their total administered

Table 6.—The rate of excretion of P^{32}-labeled metabolites by Madeira cockroaches orally injected with ronnel and certain of its hydrolysis products.[a]

	Compound Injected	Hours After Treatment				Total
		24	48	72	96	
Excreta, weight in mg.	R	9.7	3.3	1.9	1.2	16.1
	M	12.0	8.8	7.7	7.7	36.2
	C	5.3	6.8	7.6	10.0	29.7
Total μg. eliminated	R	5.28	5.96	2.95	2.41	16.60
	M	5.39	15.52	10.00	6.72	37.63
μg./mg. excreta	R	0.54	1.81	1.55	2.01	—
	M	0.45	1.76	1.30	0.87	—
Per cent total dose eliminated	R	10.4	11.8	5.8	4.8	32.8
	M	3.9	11.1	7.2	4.8	27.0

R = P^{32}-ronnel. M = P^{32}-hydrolysis products. C = Control (5 μl. propylene glycol).

[a] Data expressed as μg.-equivalents of compound per cockroach are the mean of 12 cockroaches.

Table 7.—Chromatography of tissue chloroform extracts and cockroach excreta by the normal and reverse phase systems 24 hours after oral injection of Madeira cockroaches with P^{32}-ronnel.[a]

	Rf Values of Color and Radioactive Zones at 25°–28° C.		
	Ronnel	Oxygen Analog	Ionic Metabolites
Skellysolve-B, methanol, acetic acid system			
Color standards	0.852 ± .027[b]	0.422 ± .020	0.0
P^{32}-ronnel	.860	—	—
Foregut extracts	.812	—	—
Cockroach excreta	—	—	0.0
Water, ethanol, chloroform system			
Color standards	0.049 ± .007	0.513 ± .056	0.840–0.980
P^{32}-ronnel	.050	—	—
Foregut extracts	.036	—	—
Remainder extracts	.057	—	—
Cockroach excreta	—	—	.881

[a] Rf values of the chloroform extract and excreta radioactivity are the means of two to four chromatograms for each sample; P^{32}-ronnel Rf, the mean of seven chromatograms.

[b] Standard deviation.

dose and about the same weight ratio in the excreta as the insects receiving the nontoxic derivatives.

Metabolism.—The quantity of ronnel converted to water-soluble metabolites in the tissues was determined by the difference between the fraction extracted with chloroform and the total quantity present in the homogenate as discussed under methods. The validity of this method was based on the quantitative recovery of ronnel added to control homogenates and paper chromatographic analysis of the chloroform extracts as presented in table 7. The hydrolysis products of ronnel did not partition from the water fraction of the homogenates to any appreciable extent. The data in table 7 clearly demonstrate that the radioactivity extracted with chloroform from the foregut and remainder homogenates had essentially the same Rf value as did nonlabeled and P^{32}-labeled ronnel. The oxygen analog of ronnel is soluble in chloroform but was not detected in any of the extracts by paper chromatography.

The unextracted fecal homogenates from treated cockroaches contained only the water-soluble degradation products of ronnel which were separated by paper chromatography (table 8). The largest quantities of radioactivity had Rf values corresponding to dimethyl thiophosphate and a mixture of ortho and monomethyl phosphate. Smaller quantities behaved as dimethyl-phosphate and monomethyl ronnel.

The metabolic fate of ronnel has also been investigated by Plapp & Casida (1958 a,b) employing topical application to house flies and American cockroaches. The water-partitioning metabolites produced by the house fly in a 1-hour metabolism period were approximately 90% dimethyl thiophosphate and 8% phenyl phosphates. The ronnel degradation products extracted from American cockroaches at 20 hours were qualitatively the same as those identified from the excreta in the present study except that no ortho or monomethyl phosphates were reported. These metabolites make up about 30% of the radioactivity excreted by the Madeira cockroach throughout the 96-hour posttreatment interval.

When the pattern of metabolite excretion was investigated at 24-hour intervals, a gradual but constant decrease in the relative amount of dimethyl thiophosphate and an increase of both dimethyl phosphate and monomethyl ronnel were found. This suggests that the enzyme system responsible for hydrolysis of the phenyl-phosphate bond becomes saturated or inhibited and other detoxication routes begin to play a more important role. The small quantity of monomethyl ronnel in the excreta does not completely rule out the importance of alkyl phosphate hydrolysis in the cockroach. That it never accumulates in large quantities may be caused by subsequent oxidation and hydrolysis as suggested by the ortho-monomethyl phosphate fraction excreted.

Table 8.—Chromatographic analysis of the P^{32}-labeled metabolites of ronnel present in the excreta of Madeira cockroaches.

Sample		Rf Values (25°–28° C.) and Per Cent of Total Activity on Chromatogram			
		Ortho-monomethyl Phosphate	Dimethyl Phosphate	Dimethyl Thiophosphate	Monomethyl Ronnel
Color standards	Rf	0–0.030	0.444 ± .034[a]	0.587 ± .026	0.871 ± .024
Radioactivity	Rf	0.024	0.436 ± .020	0.589 ± .021	0.870 ± .026
0–96 hours[b]	%	31.3	10.0	51.9	6.8
24 hours[c]	%	28.6	None	66.4	5.1
48 hours	%	29.8	15.6	41.7	13.0
72 hours	%	25.8	21.8	43.4	9.1
96 hours	%	30.2	22.6	35.8	11.8

[a] Standard deviation.

[b] One group of 30 cockroaches.

[c] Mean of two groups of 12 cockroaches each.

The precursors of dimethyl phosphate can be either its thio analog or the oxygen analog of ronnel. The formation of the latter has not yet been demonstrated but the slow onset of poisoning symptoms in cockroaches may coincide with the production of the oxygen analog as indicated by the build-up of dimethyl phosphate in the excreta. The conversion of a number of other phosphorothioate insecticides to their more toxic phosphates has been demonstrated as reviewed by Casida (1956).

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The Absorption and Metabolism of 3-Isopropylphenyl *N*-Methylcarbamate by Susceptible and Carbamate-Selected Strains of House Flies[1]

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Abstract

The absorption and metabolism of 3-isopropylphenyl *N*-methylcarbamate by a susceptible and a >50-fold-resistant strain of house flies (*Musca domestica* L.) were investigated by the manometric cholinesterase inhibition method. Absorption, as determined by the rate of loss of external residue, was faster in resistant than in susceptible flies. However, four times as much toxicant accumulated internally in susceptible as in resistant flies within 2 hours after application. Of the absorbed material 85% was metabolized by the resistant flies and only 23% by the susceptible ones. Treatment of resistant flies with piperonyl butoxide blocked the detoxication mechanism and resulted in a pattern of absorption and internal accumulation of toxicant roughly similar to that observed in the susceptible flies.

In a recent paper the authors reported on the development of high levels of resistance to Compound III (3-isopropylphenyl *N*-methylcarbamate) in a house fly colony, as a result of rigorous selection with this compound (Georghiou *et al.* 1961). In a comparative study of the R- (resistant) and S- (susceptible) strains, the application of p.b. (piperonyl butoxide) synergist showed that the effect of the toxicant disappeared faster in the former strain (Georghiou & Metcalf, unpublished data). This was attributed to the presence of a more efficient detoxication mechanism in the R- strain. Further evidence to this effect was sought by a study of the rates of loss of Compound III when applied to the integument of S- and R- flies and on the rates of its accumulation within the bodies of the treated insects. Determinations were also performed on flies treated with p.b. 12 hours prior to application of Compound III.

The activity of Compound III as an inhibitor of fly brain cholinesterase (I_{50} 3.4×10^{-7} M) permits the detection of as little as 0.03 μg. of this compound. This method of analysis has therefore proved especially suitable for the investigations reported here.

Materials and Methods.—The house flies employed belonged to a susceptible (S-NAIDM)[2] strain which exhibited an LD_{50} for Compound III of 1.4 μg. per female fly, and to a Compound III-selected (R-MIP)[2] strain with a resistance level of >50-fold. Only 3-day-old female flies were utilized. The methods of rearing and treatment were described elsewhere (Georghiou *et al.* 1961).

The test insects were anesthetized briefly with CO_2 and were treated on the thoracic nota with 10 μg. of Compound III in a 1-microliter volume of acetone at 85 $\pm$ 2° F. They were subsequently held at 60° F. until required for processing. A group of R- flies was treated also with 50 μg. p.b. 12 hours in advance of the insecticide. Mortality in the R- strain due to p.b. alone was zero, and to the insecticide alone <10% by the time of the final determination. Mortality was complete in S- flies, as well as in flies pretreated with p.b. These flies entered a state of paralysis within a few minutes after application of the insecticide, and death, as evidenced by complete immobilization, occurred 4 to 5 hours later. There were at least two replications of each treatment.

At various time intervals after application of Compound III, flies were removed from 60° F., anesthetized with CO_2 (if necessary) and were rinsed in two 5-ml. portions of acetone in order to remove unabsorbed insecticide. The two rinses were combined and saved for determination of the external residue. The rinsed flies were placed on filter paper until the acetone had evaporated and were subsequently macerated in a micro Potter-Elvehjem homogenizer in 5-ml. Warburg buffer (0.15M NaCl, 0.04M $MgCl_2$ and 0.025M $NaHCO_3$). The homogenate was centrifuged (1 minute at 3,000 r.p.m., 3 minutes at 4,500 r.p.m.) and the supernatant decanted and saved. Five more ml. of buffer were added and the residue was homogenized again. The two 5-ml. portions of supernatant were combined.

The amount of Compound III present externally and internally in the treated flies was determined by assaying the ChE inhibitory properties of 0.1 ml. aliquots of the acetone rinses and body tissue homogenates, respectively, and reading the results on a standard calibration curve (fig. 1). The period involved from the time of rinsing the test insects in acetone until the beginning of the ChE test was approximately 30 minutes. ChE assays were carried out by the Warburg manometric technique at 37.5° C. as already described (Metcalf & March 1949, 1950). In all determinations 2.6 ml. of enzyme preparation (three S-NAIDM homogenized fly heads per ml. of Warburg buffer), 0.3 ml. of substrate preparation (0.1M acetyl choline bromide), and 0.1 ml. of the test solution (acetone rinse or body tissue homogenate) were utilized.

The efficiency of the rinsing method was verified as follows: Treated flies were rinsed in four successive 5-ml. portions of acetone and the rinses were assayed by the ChE inhibition method for the presence of Compound III. None was found in the fourth rinse and only traces in the third. It was concluded that the first two rinses contained virtually all the detectable residue. The possibility of extracting the absorbed insecticide with acetone was investigated by homogenizing the flies in acetone or in equal amounts of acetone and Warburg buffer. This method was unsatisfactory since the homogenate assumed a jelly-like consistency. Extraction was also attempted by grinding the flies in anhydrous sodium sulfate, extracting the toxicant with chloroform, then evaporating it to dryness under a gentle stream of air and replacing it by

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[2] NAIDM, National Association of Insecticide and Disinfectant Manufacturers; MIP, *m* = isopropyl *N* = methylcarbamate.

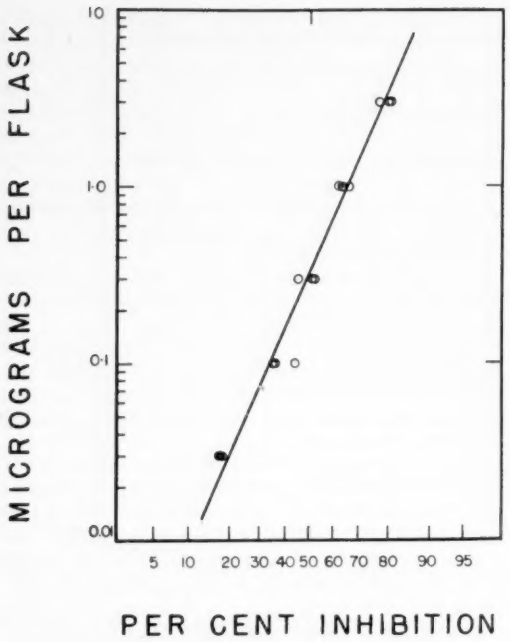

FIG. 1.—Cholinesterase inhibition standard curve for Compound III and house fly body tissue homogenate.

acetone. When aliquots of this extract were compared with equal volumes of Warburg buffer fly tissue homogenate, higher amounts of Compound III were found in the homogenate. Thus Warburg buffer was used by preference for the extraction of absorbed Compound III.

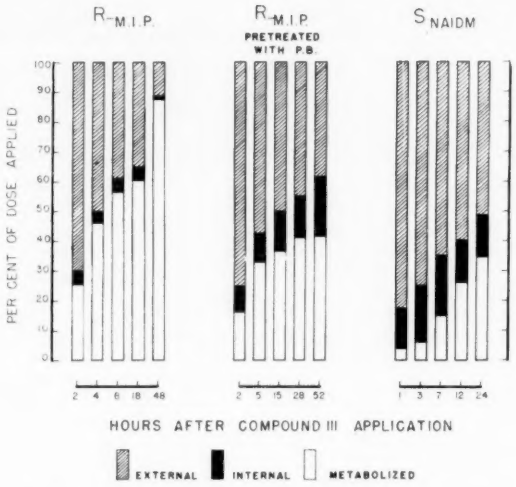

FIG. 2.—Absorption and metabolism of Compound III by R-MIP and S-NAIDM strains of house flies.

RESULTS AND DISCUSSION.—The observed and calculated results are given in table 1, fig. 2. Absorption in the R- flies proceeded at a fast rate during the first 4 hours (50% absorption), declining progressively due either to reduction of the external deposit or gradual saturation of the absorbing surfaces or both. The amount absorbed 48 hours after treatment was 88.5% of the dose applied. The rate of absorption was reduced when the flies were pretreated with p.b. The time required for 50% absorption in this case was 15 hours and the total absorbed 52 hours after treatment amounted to 61.5% of the dose applied. Absorption was the slowest in the S- flies where the 50%

Table 1.—Absorption of 3-isopropylphenyl *N*-methylcarbamate (Compound III) by resistant and susceptible house flies determined by the manometric cholinesterase-inhibition technique.

Strain	Treatment per Female Fly (T)	Hours After Compound III Application	Per Cent of Dose Applied				Per Cent Metabolized[b]
			Recovered		Calculated		
			External (E)	Internal (I)	Absorbed (T−E)[a]	Metabolized T−(E+I)[a]	$\frac{T-(E+I)}{T-E}\cdot 100$[b]
R-MIP (F_{15})	10 μg. Compound III	2	70	4.5	30	25.5	85
		4	50	3.95	50	45.05	92.1
		8	39	4.5	61	56.5	92.6
		18	35.25	4.2	64.75	60.55	93.5
		48	11.5	1.15	88.5	81.35	91.9
R-MIP (F_{15})	10 μg. Compound III preceded 12 hrs. by 50 μg. p.b.	2	75	8.75	25	16.25	65
		5	57.5	9.5	42.5	33	77.6
		15	50	13.5	50	36.5	73
		28	45	14	55	41	74.5
		52	38.5	20	61.5	41.5	69.1
S-NAIDM	10 μg. Compound III	1	82.5	13.5	17.5	4	22.9
		3	75	19	25	6	24
		7	65	20	35	15	42.9
		12	60	14	40	26	65
		24	51.5	14	48.5	34.5	71.1

[a] T = 100(%).
[b] Calculated from amount absorbed.

mark was not reached until 24 hours after application of the insecticide.

The amount of insecticide present within the insect body remained below 0.5 μg. in the case of R- flies throughout the entire 48-hour period. Flies pretreated with p.b. showed at 48 hours about 17 times greater accumulation of Compound III within the body. The rate of accumulation was the highest in S- flies where 1.35 μg. and 2 μg. were found internally 1 and 7 hours after treatment, respectively.

The calculated data on the rate of metabolism of Compound III (table 1, right-hand column) indicate that 85% of the amount absorbed by R- flies by the second hour after treatment was metabolized, as compared with about 23% metabolized by S- flies and 65% by R- flies which had been pretreated with p.b. It is interesting to note that while p.b. caused a reduction in the rate of metabolism, it did not arrest detoxication entirely. This is attributed in part to incomplete inhibition of the detoxifying enzyme(s) by p.b., since it has already been shown (Georghiou & Metcalf, unpublished data) that the degree of synergism increases as the proportion of p.b. to insecticide is increased beyond the present 5:1 ratio.

Lindquist *et al.* (1951) have shown that the rate of absorption of DDT is somewhat higher in flies surviving a treatment than in those killed by it. It is, therefore, likely that in the present work, death of the S- flies, as well as of R- flies, pretreated with p.b. resulted in lower penetration of Compound III. However, the differences between R- and S- flies observed in the present work are considerably greater than those reported by these authors for living and dead flies.

The present study indicates that resistance in the house fly strain selected with Compound III is not caused by a reduction in the absorption rate of the toxicant but by a detoxication mechanism capable of destroying it at a rate commensurate with its penetration into the insect. A similar mechanism, but of lower efficiency, is also present in susceptible flies. The remarkable synergistic effect of piperonyl butoxide for the carbamate, when applied to either susceptible or resistant strains (Georghiou & Metcalf, unpublished data), together with its action in decreasing the rate of detoxication of the carbamate, suggests that piperonyl butoxide acts by blocking the carbamate detoxication mechanism. This synergist has been assigned a similar role in pyrethrins synergism (Chamberlain 1950, Winteringham *et al.* 1955). Additional studies are in progress to further define the locus and mechanisms of action of piperonyl butoxide.

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Foliage and Soil Treatments for the Control of Flower Thrips in Outdoor Roses[1]

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ABSTRACT

Weekly spray applications of phorate reduced populations of flower thrips (*Frankliniella tritici* (Fitch)) in outdoor roses up to 3 days after application. Similar spray applications of malathion were effective for 1 but not more than 2 days. Granulated phorate applied to the soil also reduced thrips populations in rose flowers.

The flower thrips (*Frankliniella tritici* (Fitch)) often cause much damage to flowers and flower buds of outdoor roses during the spring and early summer manifested in browning of the petals and failure of affected buds to open. At Beltsville, Md., such damage is most severe early in the growing season in late May and early June. Infestations result from migrations to the rose flowers; these are soon supplemented by new generations developing in the flowers. Weekly spray applications of tartar emetic and brown sugar or weekly spray or dust applications of dieldrin, heptachlor, malathion, DDT, or lindane, and a systemic insecticide Am. Cyanamid 12008 (*O,O*-diethyl *S*-isopropylthiomethyl phosphorodithioate) failed to give control (Taylor *et al.* 1956). Control of another species of thrips on cotton has been obtained for 5 weeks after plant emergence when phorate was used as a seed treatment at the rate of 1 pound per acre (Parencia *et al.* 1957).

The present paper is a report of work conducted at Beltsville in the summer of 1957 with malathion and phor-

[1]Accepted for publication September 2, 1960.

Table 1.—The effect of phorate and malathion foliage applications at the rate of one-half pound per acre with and without soil treatments of phorate granules at the rate of 50 pounds per acre on the number of thrips per sample of five rose flowers.[a]

Soil Application	Foliage Application			
	None	Phorate	Malathion	Means[b]
	1 day after treatment			
None	162	50	82	98 a
Phorate	86	34	66	62 a
Means[b]	124 a	42 b	74 b	
	3 days after treatment			
None	82	58	88	76 a
Phorate	62	48	73	61 a
Means	72 a	53 b	81 a	
	7 days after treatment			
None	137	120	109	122 a
Phorate	97	80	79	85 b
Means	117 a	100 a	94 a	

[a] Mean number of thrips per flower sample for each treatment over the entire season.
[b] Means in the same column or row not followed by the same letter are significantly different.

ate spray applications in combination with phorate soil treatments for the control of flower thrips in roses.

METHODS.—The experiment was conducted in a completely randomized design. Each plot consisted of 10 rose plants, five each of the varieties Charlotte Armstrong and Sun King, planted in 1956.[2] Plants of the Sun King variety which were killed in the winter of 1956 were replaced in the spring of 1957 with the variety Grand'mere Jenny. These roses were selected because the large flowers provided optimum host conditions. All plots received weekly applications of 65% zineb at the rate of 2 pounds of the wettable powder per 100 gallons of spray for blackspot protection. The plants were sawdust mulched, fertilized, and kept in good growing condition by frequent irrigation.

Table 2.—Mean number[a] of thrips per sample of five rose flowers on different days after foliage applications with or without soil treatments of phorate at the rate of 50 pounds per acre.[b]

Date Sampled	No. of Days After Foliage Application	No. of Thrips Found When Soil Treatment	
		Was not Applied	Was Applied[a]
June 11	1	260 a	135 b
13	3	144	108
17	7	391 a	283 b
19	1	84	69
20	3	105	71
24	7	112 a	80 b
26	1	83	66
July 10	1	19	13
12	3	32 a	23 b
15	7	60 a	38 b
17	1	61 a	27 b
19	3	32	27
22	7	36 a	13 b
24	1	17 a	7 b
26	3	77	74
29	7	34 a	13 b

[a] Means in a row not followed by the same letter are significantly different.
[b] Phorate soil treatments applied on May 29 and July 15.

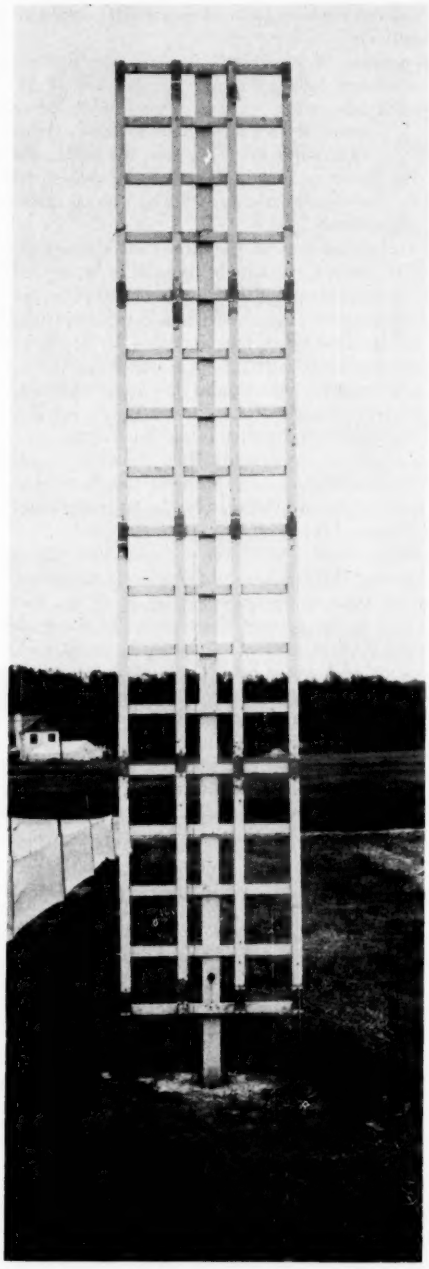

FIG. 1.—Sticky cards used in the study of flower thrips migrations.

The treatments consisted of a 3×2 factorial arrangement of malathion and phorate spray applications with or without supplementary applications of granulated phorate to the soil.

[2] The Conard-Pyle Company, West Grove, Pennsylvania, contributed the rose plants for the field experiments and made this work possible.

Malathion and phorate sprays were first applied June 10, and weekly thereafter. Malathion (25%) was used in the wettable powder form and phorate (47.5%) as an emulsion concentrate, both at the rate of one-half pound of active ingredient per 100 gallons of spray. The materials were applied with a 2-gallon tank sprayer under a pressure of 60 p.s.i. Phorate granules were applied to the plots May 29 and July 15 at the rate of 50 pounds of active ingredient per acre. The sawdust mulch was scraped away from the base of the rose plants and the material applied by hand over an area of 4 square feet.

Thrips populations were counted 1, 3, and 7 days after each foliage application. Five newly opened flowers were taken at random from the plots. The thrips were extracted by washing the flowers in a solution of Aresket 300 (Taylor & Smith 1955).

RESULTS.—*Foliage Applications.*—Phorate and malathion sprays reduced thrips populations in the flower samples taken 1 day after application. After 3 days, populations were lower in phorate-treated plots than in check plots. Malathion did not reduce thrips in flower samples taken 3 days after application (table 1).

Soil Applications.—The applications of phorate on May 29 reduced thrips populations in flowers up to July 15, or for about 6 weeks (tables 1 and 2). Each flower sample from soil-treated plots taken 7 days after a foliage application showed significant reductions. Some differences in response were seen when samples were taken 1 and 3 days after a foliage application. Sprays applied weekly in combination with the soil treatments reduced populations up to 3 days after application and may have masked the effect of soil treatments, in which case the results on soil-treated plots were not significant.

The second soil application of phorate gave similar results. The treatment reduced thrips for 2 weeks. The population thereafter was too low for an adequate sample.

Thrips Migrations.—Thrips migrations were recorded by counting the thrips caught on 2- ×5-inch sticky cards attached to a tower adjacent to the rose plots (fig. 1). Since approximately the same number of flower thrips have been caught on the cards at different heights up to 21 feet, sticky cards were placed only at the 5-foot level (Taylor & Smith 1955). The average number of thrips trapped each day is tabulated below.

Dates Exposed	*Number of Thrips*
May 31–June 4	2.0
June 4–7	14.6
June 7–10	3.6
June 10–14	7.3
June 14–24	0.3
June 24–26	2.5
June 26–July 2	1.3
July 2–9	0
July 9–17	0

The thrips caught during different periods of exposure indicated a steady increase from the last of May and reached a peak before the middle of June. Only small migrations were evident after this time. The first soil application of phorate was effective during the peak of thrips migrations; malathion and phorate sprays gave protection during this time for 1 and 3 days, respectively.

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Evaluation of Insecticides as Protectants against Pests of Stored Grain and Seeds[1]

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ABSTRACT

Results are presented from a series of tests with varying dosages of each of 36 insecticides in acetone solution sprayed on wheat for protection against adults of the rice weevil, *Sitophilus oryza* (L.), granary weevil, *Sitophilus granarius* (L.), and confused flour beetle, *Tribolium confusum* Duv., and larvae of the khapra beetle, *Trogoderma granarium* Everts. Mortalities of adult insects recorded after an exposure of 14 days and of khapra larvae after an exposure of 28 days to treated grain was the only criterion used to assess the effectiveness of insecticide deposits of 1.25, 2.5, 5, 10, 15, 25, 50, 100, and 200 p.p.m.

Malathion, owing to its present use as a grain protectant under existing residue tolerances, was considered the standard insecticide in making comparisons between compounds. Because of the resistance of khapra larvae to insecticides, only organic phosphates, but not all of those tested, were effective against all the species of test insects. Methyl parathion, phorate, parathion, and Phosdrin® (2-methoxycarbonyl-1-methylvinyl dimethyl phosphate) were more effective than malathion (10 p.p.m.) as indicated by lower dosages required for 100% mortality of all species of the test insects, while Am. Cyanamid 12009 (*O,O*-diethyl *S*-*n*-propylthiomethyl phosphorodithioate), DDVP, Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate), and Dibrom® (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate) were equally as effective. Although less efficient than malathion in preliminary tests, the desirability for further evaluation of Guthion® (*O,O*-dimethyl *S*-4-oxo-1,2,3-benzotriazin-3 (4H)-ylmethyl phosphorodithioate), ronnel, and dimethoate was indicated by the relatively high mortalities of khapra larvae exposed to wheat treated with these compounds.

By excluding khapra larvae and considering only the results from tests with the rice weevil, granary weevil, and confused flour beetle, more compounds are favored. Methyl parathion, phorate, parathion, dieldrin, Guthion, Am. Cyanamid 12009,

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Chlorthion® (*O*-(3-chloro-4-nitrophenyl) *O,O*-dimethyl phosphorothicate), Dibrom, endrin, and Phosdrin were more effective than malathion (10 p.p.m.) as indicated by lower dosages required for 100% mortality of these three species of insects. DDVP, Diazinon, and dimethoate were equally as effective, while lindane and ronnel were slightly less effective than malathion.

DDT and related compounds were among the least toxic to all insects, and Sevin® (1-naphthyl methylcarbamate) was the least effective of the 36 compounds tested, failing to cause 100% mortality of test insects of any species with the maximum dosage of 200 p.p.m.

Either contact insecticides or fumigants can be used for the chemical control of arthropods infesting stored grain and seeds. While fumigation has long been emphasized as an effective method of control of storage pests, application of insecticides for protection against infestations is a relatively new approach to the problem of preventing injury to crop seeds and keeping grain free from insects.

Parkin (1956), in his review of Stored Product Entomology, mentioned the use of pyrethrins synergized with piperonyl butoxide and a few of the chlorinated hydrocarbons for the control of stored-product insects. Residue tolerances on stored grain have not been established for any of the latter in this country, suggesting that chlorinated hydrocarbons may be unsuitable for direct application to grains intended for food or feed. However, the establishment of a residue tolerance of 8 p.p.m. for malathion on stored grain following the initial report by Lindgren *et al.* (1954) on the use of this material supports an optimistic attitude toward the potential use of other phosphorus-containing compounds as grain protectants. Less severe limitations are placed on the use of insecticides for long-term protection of crop seeds.

Insecticides on grain or seeds may give protection against storage pests by killing or repelling adults and other exposed stages, stopping or retarding the internal development of certain insects, and arresting oviposition. Obviously, procedures followed in assessment of insecticides as protectants must vary according to their mode of action. Insect mortality was used in this study as the measure of effectiveness.

It is the purpose of this paper to present results from a series of tests with varying dosages of each of 36 insecticides applied to wheat for protection against four species of stored-product insects. Results obtained are intended only to serve as a guide for the selection of insecticides to use in more detailed investigations, the minimum dosages to be applied, and the possible combination of insecticides according to specificity indicated by preliminary evaluation.

MATERIALS AND METHODS.—Insecticides tested are listed alphabetically in table 1. The chemical definitions of these compounds, except those appearing in "Common Names of Insecticides" (See Jour. Econ. Ent. **52**(2): 361–2, and **52**(5):1032), are as follows:

Am. Cyanamid 12009 = *O,O* diethyl *S*-*n*-propylthiomethyl phosphorodithioate

Am. Cyanamid 18706 = *O,O*-dimethyl *S*-(*N*-ethylcarbamoylmethyl) phosphorodithioate

Chlorthion® = *O*-(3-chloro-4-nitrophenyl) *O,O*-dimethyl phosphorothioate

Table 1.—Corrected percentage mortality of adults of the the rice weevil (RW), granary weevil (GW), and confused flour beetle (CFB) after exposure for 14 days, and larvae of the khapra beetle (KB) after exposure for 28 days to wheat sprayed with various dosages of insecticides.

Insecticide	Test Insect	Per Cent Mortality at Dosages (p.p.m.) Shown								
		1.25	2.5	5	10	15	25	50	100	200
Am. Cyanamid 12009	RW	100								
	GW	100								
	CFB	1	74	100						
	KB	12	59	97	100					
Am. Cyanamid 18706	RW	0	21	55	98	100				
	GW	1	5	49	98	100				
	CFB	0	2	3	24	69	93	100		
	KB	5	6	15	70	93	100			
Chlordane	RW	100								
	GW	37	97	99	100	99	100			
	CFB	51	76	95	99	100				
	KB	51	76	95	99	100				
Chlorthion	RW	100								
	GW	70	99	100						
	CFB	49	99	100						
	KB	0	0	13	33	48	67	88	90	94
DDT	RW	13	87	100						
	GW	5	7	48	99	100				
	CFB	0	0	11	64	89	98	99	100	
	KB	0	1	1	3	1	1	21	29	31
DDVP	RW	19	95	100						
	GW	91	100							
	CFB	0	16	98	100					
	KB	2	41	93	100					
Delnav	RW	5	18	42	88	100				
	GW	4	5	21	34	83	99	100		
	CFB	0	0	0	0	0	2	0	10	80
	KB	0	0	0	1	8	5	28	85	92
Demeton	RW	21	16	62	97	100				
	GW	11	6	35	99	100				
	CFB	0	0	2	9	21	86	100		
	KB	3	0	0	28	67	96	100		
Diazinon	RW	100								
	GW	78	100							
	CFB	2	31	96	100					
	KB	11	64	99	100					
Dibrom	RW	11	82	100						
	GW	69	100							
	CFB	0	28	100						
	KB	0	29	88	100					
Dicapthon	RW	7	23	65	94	100				
	GW	1	13	29	70	84	97	100		
	CFB	46	97	100						
	KB	98	100							
Dieldrin	RW	100								
	GW	100								
	CFB	100								
	KB	29	55	71	80	90	86	92	94	97
Dimethoate	RW	8	69	98	100					
	GW	26	92	100						
	CFB	6	29	83	100					
	KB	3	5	40	93	99	100			
Di-Syston	RW	0	39	99	100					
	GW	3	39	96	100					
	CFB	1	1	5	75	99	100			
	KB	0	1	16	61	91	100			
Dylox	RW	0	0	2	13	58	97	100		
	GW	0	7	9	52	92	100			
	CFB	0	0	1	1	3	37	87	100	
	KB	1	1	4	3	6	20	64	95	100
Endrin	RW	100								
	GW	100								
	CFB	59	99	100						
	KB	0	12	42	84	99	100			
Ethion	RW	16	18	67	96	100				
	GW	0	8	18	45	78	99	100		
	CFB	0	1	0	1	2	14	55	96	99
	KB	3	0	4	3	11	43	80	93	97
Geigy 30494	RW	69	100							
	GW	26	87	99	100					
	CFB	0	0	1	1	9	83	100		
	KB	4	17	72	96	99	99	99	100	

Table 1.—(*Continued*)

Insecticide	Test Insect	Per Cent Mortality at Dosages (p.p.m.) Shown								
		1.25	2.5	5	10	15	25	50	100	200
Guthion	RW	100								
	GW	100								
	CFB	32	98	100						
	KB	31	65	79	94	95	95	100		
Lindane	RW	100								
	GW	100								
	CFB	22	48	98	100					
	KB	6	9	4	9	9	19	94	100	99
Malathion	RW	95	100							
	GW	97	100							
	CFB	8	63	98	100					
	KB	8	27	80	100					
Methoxychlor	RW	1	10	16	32	55	72	99	100	
	GW	10	9	16	15	13	29	46	69	81
	CFB	0	0	0	1	1	0	4	7	4
	KB	0	1	0	1	1	0	3	5	5
Methyl demeton	RW	0	0	11	88	100				
	GW	0	12	87	100					
	CFB	0	0	0	4	41	94	100		
	KB	0	5	7	34	70	97	100		
Methyl parathion	RW	100								
	GW	100								
	CFB	100								
	KB	100								
Monsanto CP7769	RW	0	0	0	62	95	100			
	GW	0	0	2	59	77	99	100		
	CFB	0	0	1	2	24	77	100		
	KB	0	1	0	2	38	95	100		
Parathion	RW	100								
	GW	100								
	CFB	100								
	KB	100								
Phorate	RW	100								
	GW	100								
	CFB	100								
	KB	96	100							
Phosdrin	RW	100								
	GW	100								
	CFB	7	29	100						
	KB	64	89	100						
Phostex	RW	0	27	20	23	51	99	100		
	GW	0	6	4	5	9	20	93	100	
	CFB	0	0	0	0	0	0	16	89	100
	KB	1	0	0	0	0	0	5	62	98
Ronnel	RW	91	93	99	100					
	GW	88	94	99	100					
	CFB	2	21	33	96	100				
	KB	17	41	64	94	97	100	99	100	
Sevin	RW	0	0	0	2	6	18	57	79	98
	GW	4	7	15	12	15	26	58	85	99
	CFB	0	0	0	1	0	1	0	4	40
	KB	1	5	7	6	5	12	13	15	27
Shell BAS 4092	RW	100								
	GW	100								
	CFB	0	1	7	51	91	100			
	KB	22	81	100						
Shell SD 3562	RW	100								
	GW	99	99	100						
	CFB	2	0	2	4	10	35	85	100	
	KB	35	7	17	7	21	51	86	100	
Synergized Pyrethrum[a] (in Pyrenone O.F. 75–7.5)	RW	68	100							
	GW	95	100							
	CFB	1	10	66	89	97	99	100		
	KB	10	16	74	88	90	99	100		
TDE	RW	0	7	32	76	95	100			
	GW	0	0	0	7	9	21	88	100	
	CFB	2	3	1	2	3	0	5	32	50
	KB	2	1	0	0	0	0	1	8	23
Trithion	RW	2	19	40	91	100				
	GW	10	19	35	60	94	100			
	CFB	1	2	3	5	4	13	71	88	100
	KB	—	9	6	23	21	66	99	100	

[a] Dosages are in terms of p.p.m. pyrethrins.

Delnav®=2,3-*p*-dioxanedithiol *S,S*-bis (*O,O*-dimethyl phosphorodithioate)

Diazinon®=*O,O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate

Dibrom®=1,2-dibromo-2,2-dichloroethyl dimethyl phosphate

Di-Syston®=*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate

Dylox®=dimethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate

Geigy 30494=*O,O*-dimethyl *S*-2,5-dichlorophenylmercaptomethyl phosphorodithioate

Guthion®=*O,O*-dimethyl *S*-4-oxo-1,2,3-benzotriazin-3 (4H)-ylmethyl phosphorodithioate

Monsanto CP 7769=hexaethyl (ethylthiomethylidyne) triphosphonate

Phosdrin®=2-methoxycarbonyl-1-methylvinyl dimethyl phosphate

Phostex®=bis(dialkoxyphosphinothioyl) disulfides (alkyl ratio 25% isopropyl, 75% ethyl)

Sevin®=1-naphthyl methylcarbamate

Shell BAS 4092=2-(benzyloxycarbonyl)-1-methylvinyl dimethyl phosphate

Shell SD 3562=2-dimethylcarbamoyl-1-methylvinyl dimethyl phosphate

Trithion®=*S*-[*p*-chlorophenylthio)methyl] *O,O*-diethyl phosphorodithioate

The synergized pyrethrum formulation (Pyrenone® O.F. 75–7.5) was a mixture containing 75% piperonyl butoxide and 7.5% pyrethrins in solution.

Insecticide in acetone was used in all spray preparations. With the exception of 90% methoxychlor oil concentrate and compounds obtained in the technical form (DDVP, demeton, Dylox, lindane, malathion, parathion, Phosdrin, Phostex, Sevin, Shell BAS 4092, Shell SD 3562, and Trithion), solution concentrates served as the source of insecticides for preparation of stock solutions in acetone. Freshly made stock solutions were diluted as necessary to contain the amounts of insecticide needed in 10 ml. of spray for the desired deposits of 1.25, 2.5, 5, 10, 15, 25, 50, 100, and 200 p.p.m. when applied to 500-gram samples of wheat.

A filling hopper ordinarily used in grain testing for weight per bushel measurements held wheat to be sprayed. Spray was applied into the grain stream with a *Binks "Wren" Air-Brush* made by the Binks Manufacturing Company, Chicago, Illinois. The air-brush was equipped with constant air pressure. The sliding plate valve at the bottom of the filling hopper was opened only enough to allow the flow of grain to coincide with the application of spray. A continuous flow was maintained by agitating the grain slowly over the valve opening at the bottom of the filling hopper with a laboratory stirrer. The moisture content of White Federation wheat as determined with an *"Elf" Universal Moisture Tester* ranged from 9% to 11%. Moisture was not adjusted for uniformity between tests.

Sprayed wheat was left overnight in open polyethylene bags at room temperature before blending 5 minutes in a Twin Shell Blender of 8-quart capacity. After blending, four samples to be used for toxicity tests were removed from each lot of wheat.

One lot of sprayed wheat represented one treatment replicate; treatments were replicated three times in all experiments. Unsprayed wheat was used in all tests as control standards for comparison.

The effectiveness of insecticide deposits on wheat was tested by confining 100 adults each of the rice weevil,

Sitophilus oryza (L.), the granary weevil, *Sitophilus granarius* (L.), and the confused flour beetle, *Tribolium confusum* Duv., and 100 larvae of the khapra beetle *Trogoderma granarium* Everts, in approximately 50 grams of wheat from each treatment replicate. Samples for toxicity tests were held in cloth-covered wide-mouth glass jars. The wheat was screened and mortalities recorded after an exposure of 14 days for the rice weevil, granary weevil, and confused flour beetle and 28 days for khapra larvae. Test samples were held in a room maintained at 80° F. and 60% ± 5% relative humidity.

Granary weevils, rice weevils, and confused flour beetles were reared as previously described by Strong & Sbur (1960). They were used for toxicity tests 14 days after the first adults appeared in cultures, giving test insects with a mean adult age of 7 days and a range in age of 1 to 14 days. Khapra larvae were reared on ground wheat at a temperature ranging from 90° to 95° F. and a relative humidity of 40% ± 2%. Mature larvae were selected at random for toxicity tests.

RESULTS AND DISCUSSION.—Mortality of test insects was the only criterion used to measure the effectiveness of insecticide deposits in protecting treated wheat against storage pests. Although the mortality of insects in unsprayed control samples of wheat was generally low, results (in nearest whole numbers) given in table 1 were corrected by Abbott's formula (1925). It should be noted that when the last figure in a row indicates 100% mortality of insects for a particular dosage of insecticide in table 1, this mortality was recorded for all of the dosages applied at a higher level. Repeated mortalities of 100% were not listed intentionally so the tabulations would have a graphic appearance. The lack of tabular data indicates at a glance those materials which were most effective.

Since insect mortality was the measure of effectiveness in this study, malathion was considered the standard for use in making comparisons between insecticides. Malathion can be used against insect infestations in stored grain under existing residue tolerances and is a toxic material.

In general, with the notable exception of dicapthon, khapra larvae were less susceptible to insecticides than the other three species of insects tested. Because of the resistance of khapra larvae to insecticides, when comparing results with protectants against the four species of insects only organic phosphates, but not all of those tested, were considered in further tests. Four compounds—methyl parathion, phorate, parathion, and Phosdrin—were more effective than malathion as indicated by lower dosages required for 100% mortality of the four species of insects tested. Four insecticides, Am. Cyanamid 12009, DDVP, Diazinon, and Dibrom, were equally as effective as malathion. In addition to the organic phosphates mentioned above, the relatively high mortalities of khapra larvae exposed to Guthion, ronnel, and dimethoate indicate the desirability for further evaluation of these materials as grain and seed protectants against stored-product insects.

By excluding khapra larvae and considering only the three species of insects tested that are found generally throughout this country, more compounds, including several chlorinated hydrocarbons, are favored as desirable for further evaluation. Ten insecticides—methyl-parathion, phorate, parathion, dieldrin, Guthion, Am. Cyanamid 12009, Chlorthion, Dibrom, endrin, and Phosdrin—were more effective than malathion as indicated by lower dosages required for 100% mortality of the confused flour beetle, the rice weevil, and the granary weevil; three insecticides—DDVP, Diazinon, and dimethoate—were equally as effective as malathion; and two compounds, lindane and ronnel, were slightly less effective.

Differences between species in susceptibility to insecticides is indicated by the data presented in table 1. This information may be of value in selecting materials to use in combination against several species of stored-product insects. Otherwise, it appears logical to base dosage requirements on results obtained from tests with insects most difficult to control.

It is of interest to note similarities between results from this study and those obtained by Vincent & Lindgren (1957) with topical application of insecticides to three species of stored-product insects. In both studies, insecticides most effective against khapra larvae were all organic phosphates; chlorinated hydrocarbon insecticides were relatively ineffective against khapra larvae. DDT and related compounds were among the least toxic to all insects tested. Several chlorinated aryl hydrocarbons were among the more effective compounds tested when comparisons are made excluding khapra larvae.

Insecticides such as Sevin, the least effective of the 36 compounds tested, probably should not be evaluated further unless comparisons are to be made in tests designed to evaluate such materials as repellents to insects infesting stored grain and seeds.

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Hydrolysis of Certain Organophosphate Insecticides by House Fly Enzymes[1]

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Abstract

Insects are known to degrade readily most organophosphate insecticides *in vivo*. *In vitro* hydrolysis of certain organophosphates was effected by insect homogenates, particularly in the presence of manganic ion as an activator. Purification of house fly esterases showed that the enzymes hydrolyzing the dialkyl phosphoric anhydrides can be at least partially separated from the choline, aliphatic and aromatic esterases, and that more than one house fly esterase acts on the phosphates. The organophosphate substrate specificity is considered for homogenates of several insects and mammalian tissues. These organophosphate-hydrolyzing esterases of insects are discussed with respect to the species—specificity in poisoning by organophosphates and the acquired resistance of house flies to certain organophosphates

Enzymes involved in detoxification of organophosphate cholinergic agents have been extensively investigated with mammalian plasmata and certain other mammalian tissues (Adie & Tuba 1958, Aldridge 1953, Augustinsson & Heimburger 1954, Cook *et al.* 1958, Main 1960, Mounter *et al.* 1955, and many others). Despite the wide scale use of organophosphates as insecticides, little information is available on the insect enzymes effecting their hydrolysis. Insect homogenates active in organophosphate detoxification have been demonstrated with house flies (Asperen & Oppenoorth 1960), honey bees (Metcalf *et al.* 1956), rice stem leafhoppers (Kojima & Ishizuka 1960) and cockroaches (O'Brien 1957). These insect homogenates are generally much less active than mammalian tissue homogenates in organophosphate hydrolysis. Radio-tracer studies on the rate of *in vivo* detoxification of organophosphate insecticides have clearly shown a more rapid degradation in mammals than in insects (March *et al.* 1956, Krueger & O'Brien 1959, Krueger *et al.* 1960). It has been postulated that the activity and/or specificity of these detoxifying esterases may be involved in the species selectively in poisoning by organophosphates (Arthur & Casida 1957, Kojima & Ishizuka 1960, Krueger & O'Brien 1959, Krueger *et al.* 1960, March *et al.* 1956, O'Brien 1957, O'Brien *et al.* 1958) and in the acquired resistance of house flies to certain of these insecticides (Asperen & Oppenoorth 196 , March 1959, Oppenoorth & Asperen 1960).

The activity of insect homogenates was compared with those of certain mammalian tissues in catalyzing the hydrolysis of a variety of organophosphates. A partial purification of the house fly enzymes hydrolyzing tetraethyl pyrophosphate (TEPP), diisopropyl fluorophosphate (DFP) and 2,2-dichlorovinyl dimethyl phosphate (DDVP) was effected and the properties of these enzymes were investigated.

Materials and Methods.—*Tissues and Compounds.*—The "normal" house fly, *Musca domestica* L. (Chemical Specialties Manufacturers Association (CSMA)-1948 strain), was obtained from the Wisconsin Alumni Research Foundation Insecticide Laboratory, Madison, Wisconsin. An organophosphate-resistant strain of house flies, designated as the Orlando "Tropical P" strain (LaBrecque *et al.* 1958, Mengle & Casida 1960, Wilson *et al.* 1959), was also employed and was reared through two generations in our laboratory without insecticide pressure. Adult male and female house flies were used 3 days after emergence with both of the above strains.

Adult American cockroaches (*Periplaneta americana* (L.)), black carpet beetle larvae (*Attagenus piceus* (Oliv.)), adult large milkweed bugs (*Oncopeltus fasciatus* (Dall.)) and adult honey bees (*Apis mellifera* L.) and mixed larvae and adult grain mites (*Acarus siro* L.) were also studied. All of the arthropods were reared according to standard laboratory procedures except the honey bees which were from a colony held indoors but allowed to forage outdoors.

Rabbit plasma was prepared from heparinized blood pooled from four animals. Rat livers were obtained from 250-gm. female white rats.

The organophosphates used other than TEPP, DFP and DDVP were: Dipterex® (dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate), malathion, parathion, para-oxon, Dibrom® (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate), Chlorophan® (*O,O*-dimethyl 1-methoxy-2,2-dichlorovinyl phosphate), and Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate).

All of the above compounds were of the highest purity obtainable from the manufacturers. Para-oxon was further purified according to the method of Aldridge (1953).

Assay of Esterase Activity.—Esterase activity was usually determined indirectly by CO_2-evolution from a bicarbonate buffer (0.0357 M $NaHCO_3$) in an atmosphere of 5% CO_2 and 95% N_2 at 38° C. in 10-ml. Warburg flasks. The enzyme in 0.40 ml. was added to the side arm of the flask, and the substrate in 1.60 ml. to the main compartment. The amount of tissue used in each Warburg flask was standardized at 40 mg. for the homogenates of whole insects or of rat liver and at 200 μl. for heparinized rabbit plasma. The flasks were gassed for 6 min. while shaking at 38° C., the substrate tipped and at 5-min. intervals thereafter manometer readings were recorded. Esterase activity was expressed as b_{30} (μl. CO_2 evolved per 30 min.) after correction for non-enzymatic hydrolysis of the substrates and endogenous liberation of CO_2 by the enzyme in the absence of substrate. When the rate of CO_2 evolution

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decreased within the 30-min. period, only the linear portion of the time-CO_2 liberation relationship was used in deriving the b_{30}.

Emulsions were used for the substrates which were not water soluble at 0.01 M. The final concentration of Triton X-100 (Rohm and Haas Co., Philadelphia, Pennsylvania, U.S.A.) and ethanol used in preparing these emulsions was 0.5% and less than 1%, respectively.

The activating effect of certain divalent cations was investigated by introducing the same level of activator into the side arm and the main compartment of the flask so that no dilution of activator occurred on tipping. Chloride salts of these cations were initially dissolved in the bicarbonate buffer which had been gassed with 5% N_2 and 95% CO_2 to minimize precipitation of the cations. A 30-min. preincubation period of activator and enzyme at 25° C. was used prior to placing the flasks in the Warburg at 38° C. for equilibration. Inhibitor ions were studied at 1×10^{-4} M by the same procedure used for the activation studies, and in all inhibitor studies Mn^{2+} was present at 1×10^{-3} M in order to enhance the esterase activity for increased accuracy in measurement.

Para-oxon hydrolysis was followed colorimetrically as well as manometrically. Whole flies were homogenized in distilled water, filtered through cheesecloth to remove debris and, after the addition of appropriate co-factors, a 30-min. preincubation at 25° C. was allowed for possible activation. The brei at pH 6.8 was then incubated with 1×10^{-2} M para-oxon at 38° C. with shaking. Two-ml. aliquots were removed from the total 10-ml. volume and each aliquot was mixed with 2.0 ml. acetone and allowed to stand at 25° C. for 30 min. The precipitated proteins were then removed by filtration and 2.0 ml. 0.1 M pH 8.9 phosphate buffer was added and the free *p*-nitrophenate ion determined immediately at 400 mμ. Boiled fly brei was used as a control in order to correct for non-enzymatic hydrolysis.

Fractionation of House Fly Esterases.—Homogenates of whole house flies were prepared at 20% w/v in the bicarbonate buffer with a glass homogenizer. The debris was removed by filtering through cheesecloth to yield the fraction designated as "whole homogenate." The "soluble fraction" was the supernatant after centrifugation at 100,000 × g. and 5° C. for 1 hour.

Alcoholic fractionation was accomplished by cooling the soluble fraction to 0° C., adding a small amount of redistilled 95% ethanol and then lowering the temperature to −5° C. at which time the rest of the ethanol was added dropwise with constant stirring. After 30 min. at −5° C. the precipitate was separated from the alcohol-soluble fraction by centrifuging at 18,000 × g. for 10 min. at 5° C. The precipitate was then resuspended in cold buffer and the operation repeated with the alcohol-soluble fraction by adding to it even higher alcohol concentrations.

Ammonium sulfate fractionation was accomplished in an almost identical manner to the alcoholic fractionation except that the appropriate amounts of solid ammonium sulfate were added very slowly with vigorous stirring at 0° C.

RESULTS AND DISCUSSION.—*Activation by Divalent Cations.*—The hydrolysis of TEPP, DFP and DDVP by the house fly soluble fraction was greatly enhanced by 1×10^{-3} M Mn^{2+} and somewhat increased by a comparable level of Co^{2+}, while this concentration of Ba^{2+}, Ca^{2+}, Mg^{2+} or Sr^{2+} was without effect on these enzymes (table 1). The difference in the degree of activation of the hydrolysis with the three different substrates indicates the involvement of more than a single enzyme. The necessity for high Mn^{2+} and Co^{2+} concentrations for activation is in agreement with the studies of Mounter *et al.* (1953) on the hog kidney enzymes hydrolyzing DFP. The effect of the cations on DFP hydrolysis by the house fly enzymes was more similar to that for mammalian kidney and liver preparations (Mounter 1955, Mounter *et al.* 1953, 1955) than for mammalian serum esterases hydrolyzing this and other organophosphates (Augustinsson & Heimburger 1955). The 19-fold activation of DDVP hydrolysis with Mn^{2+} and the house fly esterases is higher than the usual degree of activation noted with cation activators and mammalian esterases hydrolyzing such organophosphates. Since Mn^{2+} enhanced the activity of the fly enzymes on all the substrates to the extent that their hydrolysis could be accurately measured, this activator at 1×10^{-3} M was used in all subsequent studies.

Table 1.—The effect of metallic ions on the hydrolysis of TEPP, DFP and DDVP by house fly homogenates.[a]

Substrate	No Co-Factor	Mn^{2+}(M)			Co^{2+}(M)		
		1×10^{-3}	1×10^{-5}	1×10^{-7}	1×10^{-3}	1×10^{-5}	1×10^{-7}
TEPP	55	225	60	55	50	45	45
DFP	37	132	45	43	95	39	39
DDVP	8	154	35	9	15	9	6

[a] Activity as b_{38} at 38° C. with 40 mg. of house fly fresh weight. All substrates were at 1×10^{-2} M. (Mean of 2–4 replicates in separate runs.)
No activation or inhibition at 1×10^{-2} M with Ba^{2+}, Ca^{2+}, Mg^{2+} and Sr^{2+}.

Purification of the House Fly Esterases Hydrolyzing TEPP, DFP and DDVP.—All of the activity of whole fly homogenates for hydrolyzing TEPP, DFP and DDVP was recovered in the soluble fraction from 100,000 × g. centrifugation. Dialysis against distilled water yielded no loss in activity or change in the efficiency of divalent cation activators compared with the undialyzed soluble fraction. On addition of ethanol to the soluble fraction, usually more than 70% of the total activity against the three phosphate esters was recovered in the precipitate formed on increasing the alcohol content from 40% to 70%. Less than 5% of the total activity was precipitated at alcohol concentrations in excess of 70%. The distribution of activity and degree of purification with alcohol fractionation are indicated in table 2. The per cent activity recovered and degree of purification were higher for TEPP and DDVP than for DFP. With these first two substrates more than 100% of the original activity of the homogenate was recovered in the protein fractions precipitated with alcohol, possibly attributable to removal of proteins resulting in an increase in the efficiency of the activator (Mounter *et al.* 1955). Further fractionation with alcohol concentrations between 60% and 70% failed to yield a higher degree of purification. Assays of the alcoholic fractions on carboxylic esters (table 2) showed that the esterases hydrolyzing the phosphates were precipitated at a higher alcohol concentration than the majority of the activity with any of the choline, aliphatic and aromatic esters studied. It is probable that the aliphatic and aromatic esters were hydrolyzed by more than a single

Table 2.—Substrate specificity of fractions obtained from whole fly homogenate by alcoholic precipitation. (Mean of 2–4 replicates in separate runs.)

Property of Fraction		Fraction					
		Whole Homogenate	Soluble	20%	40%	60%	70%
Per cent of total protein[a]		100	30	3	9	6.6	2
Activity in hydrolysis of 1×10^{-2} M phosphate ester in presence of 1×10^{-3} M Mn^{2+}							
DFP	Degree of purif.	1	3.1	0	0.6	3.1	26
	% act. recovered	100	100	0	15	21	52
TEPP	Degree of purif.	1	3.1	0	0.4	5.5	55
	% act. recovered	100	100	0	3.6	36	108
DDVP	Degree of purif.	1	3.1	0	0.5	3.5	47
	% act. recovered	100	100	0	14	23	91
b_{50}/mg. protein with 0.01 M carboxylic ester							
Acetyl choline		12	12	37	9.6	8.8	0
Propionyl choline		10	12	44	7.7	8.8	0
Butyryl choline		14	12	23	9	0	0
Triacetin		16	11.5	17	9.6	6.6	15
Phenyl acetate		44	88	56	13	6.6	15
o-nitrophenyl acetate		43	136	65	23	40	35

[a] Original protein content of 20% whole fly homogenate was 43 mg./ml. based on the biuret method (Robinson & Hogden 1940).

enzyme (Asperen 1959). The possibility remains that a portion of the activity in hydrolysis of the aromatic esters might be caused by the enzymes hydrolyzing the phosphate esters. Such a possibility would be consistent with the work of Metcalf *et al.* (1956) on honey bee A-esterase and Aldridge (1953) and others (Augustinsson & Casida 1959) studying the mammalian enzymes involved in both phosphate and carboxylic ester hydrolysis.

Ammonium sulfate fractionation yielded less than a ten-fold purification of the TEPP, DFP and DDVP hydrolyzing esterases since the activity per mg. protein changed little from the soluble fraction through a series of protein precipitations resulting from 20%, 30%, 40%, 50%, 60% and 70% saturation with this salt. Sodium cholate at 1 mg./10 mg. protein did not affect the ammonium sulfate fractionation in any way. An attempt was made to combine the alcohol and ammonium sulfate fractionation procedures by using the soluble material from 50% ethanol, dialyzing this supernatant and then fractionating with ammonium sulfate. Less than 4% of the activity against TEPP, DFP and DDVP was precipitated with 50% saturated ammonium sulfate. The majority of the activity appeared in the fraction precipitating between 50% and 70% saturation and showed a twofold purification with DFP without loss of total enzyme activity, while with TEPP and DDVP a loss in both purity and enzyme activity occurred.

Sensitivity of the Fly Esterases Hydrolyzing TEPP, DFP and DDVP to Inhibitors, Heat and Cold.—The fly soluble fraction was sensitive to inhibition by 1×10^{-4} M *o*-iodosobenzoic acid, *p*-chloromercuribenzoic acid, mercuric chloride and cupric chloride but was not inhibited by this level of sodium cyanide and sodium fluoride. Complete inhibition resulted with cupric chloride, and 80% to 100% inhibition with mercuric chloride even though both salts partially precipitated under the assay conditions. The soluble benzoic acid derivatives yielded 80% to 100% inhibition with the *p*-chloromercuri analog and 15% to 45% inhibition with the *o*-iodoso analog. No great difference in sensitivity to these inhibitors occurred with the three substrates.

Partially purified enzyme from the 60% to 70% alcoholic fractionation showed a 50% loss in esterase activity against TEPP and DDVP and a 41% loss against DFP on holding in bicarbonate buffer at pH 7.2 for 60 min. at 38° C. before assay. A similar enzyme preparation in bicarbonate buffer held 14 days at 5° C. showed 60%, 78% and 82% loss, respectively, with DFP, TEPP and DDVP as substrates. Whole flies held several weeks at −20° C. were comparable to fresh flies in the activity of the fly esterases hydrolyzing these phosphates.

Substrate Specificity of Esterases Hydrolyzing Insecticidal Organophosphates.—The activity of homogenates of five insect and one mite species and of rat liver and rabbit plasma were compared in the presence of 1×10^{-3} M Mn^{2+} (table 3). The house fly showed the highest activity of the arthropod homogenates, with the notable exception of the substrate Chlorophan which was only hydrolyzed by the black carpet beetle larvae. It is interesting that with the house fly the enzymatic degradation of Dibrom was not activated by Mn^{2+} while activation occurred with the other dimethyl phosphate, DDVP, and with TEPP and DFP. The house fly esterases hydrolyzing Dibrom were present only in the soluble fraction in a similar manner to those hydrolyzing the three other organophosphates. Assays on the separated head, thorax and abdomen of house flies showed similar distribution of activity in hydrolyzing TEPP, DDVP and DFP. By comparing activities on a fresh weight basis, the abdomen was about three times more active than either the head or thorax.

Table 3.—*In vitro* organophosphate hydrolysis by insect and mammalian tissues.[a]

Substrate	Cheese Mite	American Cockroach	Milkweed Bug	Black Carpet Beetle Larvae	House Fly	Honey Bee	Rat Liver	Rabbit Plasma
TEPP	100	60	100	82	225 (55)[b]	35	220	160
DFP	60	14	30	66	132 (37)	10	340	191
DDVP	70	19	46	70	154 (8)	42	130	60
Dibrom	50	0	0	0	40 (40)	0	37	55
Chlorophan	0	0	0	30	0 (0)	0	30	60
Malathion	0	0	0	0	0 (0)	0	50	0
Phosdrin	0	0	0	0	0 (0)	0	0	0
Dipterex	0	0	0	0	0 (0)	0	0	0
Parathion	0	0	0	0	0 (0)	0	0	0
Para-oxon	0	0	0	0	0 (0)	0	0	350

[a] Activity as b_{30} at 38° C. with 40 mg. insect or liver fresh weight or 200 µl. plasma per flask in the presence of 1×10^{-3} M Mn^{2+}. All substrates were at 1×10^{-4} M. Mean of 2 to 4 replicates in separate runs.
[b] Values in parenthesis represent b_{30} in absence of Mn^{2+}.

The topical LD_{50} as µg. toxicant/gm. insect varies more than 75-fold between the susceptible adult house fly and the tolerant black carpet beetle larva when DFP and Dipterex are bioassayed (Krueger & Casida 1957). This species difference in susceptibility is not correlated with the ability of homogenates of house flies and beetle larvae to hydrolyze DFP and Dipterex (table 3) indicating that these enzymes are probably not associated with the natural tolerance of the black carpet beetle larvae.

Rabbit plasma is known to be active in hydrolysis of para-oxon (Aldridge 1953, Mounter 1954) and several other organophosphates (Augustinsson & Heimburger 1954). In the present study rabbit plasma was assayed in the presence of Mn^{2+}, an ion which may slightly inhibit the activity of these esterases (Mounter 1954). Rabbit plasma catalyzed the hydrolysis of TEPP, DFP and para-oxon but not of malathion (Augustinsson & Heimburger 1954, and table 3) and was also active with DDVP, Dibrom and Chlorophan (table 3). Of the tissues examined, only rat liver was active in malathion hydrolysis (table 3). The activity of rat liver (Cook *et al.* 1958) and inactivity of fly homogenates (Weidhaas 1959) on malathion have been previously reported.

Hydrolysis of Para-oxon by House Fly and Honey Bee Homogenates.—Contrary to the results of Metcalf *et al.* (1956), no hydrolysis of para-oxon or parathion was observed in manometric studies with honey bee homogenates (table 3). Repeating these experiments with the same buffer system used by these workers also yielded no CO_2-evolution with para-oxon and parathion substrates.

The *in vitro* degradation of para-oxon as measured by release of *p*-nitrophenate ion in the presence of house fly and honey bee homogenates is shown in table 4. This method for measuring para-oxon hydrolysis is at least 50 times more sensitive than the manometric method used routinely in these investigations. The extent of enzymatic degradation of para-oxon by house flies was increased threefold by 1×10^{-3} M Mn^{2+}. The most effective activator was Mn^{2+} since the "complete co-factor" (composition of co-factor mixture given in table 4) containing Mn^{2+} yielded the same degree of activation as with Mn^{2+} alone, and the other co-factor, Co^{2+}, yielded only a twofold activation. Comparison of the amount of para-oxon degraded by homogenates of normal and organophosphate-resistant house flies showed that similar para-oxon hydrolysis occurred with the strains both in the presence and absence of Mn^{2+}, despite the fact that this Orlando "Tropical P" strain was 6.7 times as resistant to topically applied para-oxon (Mengle & Casida 1960). Honey bee homogenates were less than one-half as active as house fly homogenates when compared on a fresh weight basis and were only activated twofold by the "complete co-factor" mixture (table 4).

Asperen & Oppenoorth (1960) found in studies on para-oxon degradation that an excellent correlation existed between the level of resistance to para-oxon and the ability of homogenates from these fly strains to degrade para-oxon.

The data in tables 3 and 4 show that approximately 3.5 µg. of para-oxon and 40 µg. of DDVP (by colorimetric and manometric procedures, respectively) are degraded by each fly in 30 min. at 38° C. which is approximately 8,000 to 55,000 times the level of organophosphate destroyed at 27° C. as reported by Asperen & Oppenoorth (1960). This large discrepancy cannot be explained by the temperature difference alone, but may be partially caused by differences in saturation of the degrading enzymes by

Table 4.—Para-oxon hydrolysis by house fly and honey bee homogenates.[a]

Homogenate	µG. Para-oxon/2 Hrs./Insect[b]
Normal house fly	
No co-factor	14
Complete co-factors[c]	44
Mn^{2+} 1×10^{-3} M	44
Co^{2+} 1×10^{-3} M	28
Mg^{2+} 1×10^{-3} M	14
Resistant house fly ("Tropical P")	
No co-factor	14
Mn^{2+} 1×10^{-3} M	48
Honey bee	
No co-factor	14
Complete co-factors[c]	28

[a] Mean of 2 to 4 replicates in separate runs with degree of para-oxon hydrolysis based on *p*-nitrophenol determined colorimetrically.
[b] Weight of individual insects calculated as 20 mg. for house fly and 50 mg. for honey bee.
[c] Adenosine triphosphate, diphosphopyridine nucleotide in both its oxidized and reduced forms, and triphosphopyridine nucleotide each at 1×10^{-4} M and Co^{2+}, Mg^{2+} and Mn^{2+} each at 1×10^{-3} M.

the organophosphate since the concentrations of para-oxon and DDVP used in this investigation were 70,000 to 500,000 times that used by the above workers. Lowering the concentration of para-oxon from 1×10^{-2} M to 1×10^{-3} M under the conditions described in table 4 resulted in no detectable enzymatic hydrolysis. Oppenoorth & Asperen (1960) more recently have stated that certain strains of house flies resistant to parathion and Diazinon show nearly complete saturation of the degrading enzymes at a substrate concentration of the oxygen analog of 1×10^{-8} M. It would appear that the techniques used in the present study and those used by Asperen and Oppenoorth were not measuring the same enzyme and/or reaction.

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Control of the Tarnished Plant Bug, *Lygus lineolaris*, on Birdsfoot Trefoil Grown for Seed[1]

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Abstract

Studies were undertaken to determine the effect of the control of tarnished plant bug, *Lygus lineolaris* (P. de B.), on birdsfoot trefoil, *Lotus corniculatus* L., seed production. The study of populations of the tarnished plant bug in six fields in New York throughout the growing season indicated that a large increase in numbers of this insect occurred toward the last of June. Insecticide applications were made experimentally in mid-June to prevent this build-up.

A method was developed whereby the seed yields in pounds per acre could be estimated by counting the number of legumes present in 20 one-square-foot areas.

Treatments with aldrin, parathion, DDT, and toxaphene significantly increased seed yields from birdsfoot trefoil when applied to control the tarnished plant bug. These increases in seed yields occurred in experiments having check plots that averaged 16 or more tarnished plant bugs in 25 net sweeps. However, in some untreated plots the tarnished plant bugs were considerably more numerous.

Prominent among the insects injurious to birdsfoot trefoil, *Lotus corniculatus* L., are several species belonging to the family Miridae. These are the tarnished plant bug, *Lygus lineolaris* (P. de B.); the alfalfa plant bug, *Adelphocoris lineolatus* (Goeze); the rapid plant bug, *Adelphorcoris rapidus* (Say); *Plagiognathus politus* Uhler, and *Plagiognathus chrysanthemi* (Wolff).

Neunzig (1955), Neunzig & Gyrisco (1955), and Neunzig *et. al.* (1958) reported that the alfalfa plant bug and the rapid plant bug feed upon the mature floret buds and the blossoms of trefoil causing them to blast while the tarnished plant bug feeds upon the partially mature floret buds causing these to blast, also. The type of injury caused by *Plagiognathus* spp. is not as yet known although it is probably similar to that caused by the other mirids.

MacCollom (1958) and Ridgway & Gyrisco (1959) report that trefoil seed yields were increased by the application of insecticides. Their results seemed to justify further experiments to determine the effect of mirids on seed yields. Therefore, in 1958–59 four experiments in three widely separated birdsfoot trefoil growing areas were conducted for the control of the tarnished plant bug and other mirids. In this paper the tarnished plant bug is considered separately because it was the most common mirid found on birdsfoot trefoil. All the other plant bugs are considered as a group and referred to as "other mirids."

Methods and Materials.—Since the timing of sprays at peaks in populations of the bugs was thought desirable, levels of populations were determined throughout the 1958 and 1959 growing seasons by taking 100 net sweeps at approximately weekly intervals with a 15-inch-diameter insect-collecting net at six different locations. The insect counts from the fields for each week were averaged and are presented graphically in figure 1. It should be emphasized that near the end of June there was a sharp increase in the total number of tarnished plant bugs present in birdsfoot trefoil. This increase results from the first generation nymphs which hatched from eggs laid by overwintering females. Most of the insecticidal treatments applied in these experiments were applied in mid-June to prevent this sharp increase in population numbers.

Endrin, aldrin, DDT, toxaphene, parathion, and malathion were applied alone or in various combinations with each other as emulsions to 20- by 50-foot plots that were

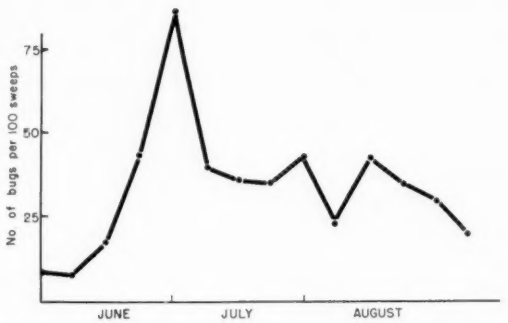

Fig. 1.—Mean numbers of tarnished plant bugs collected at approximately weekly intervals from birdsfoot trefoil in six locations in New York. 1958–1959.

randomized within blocks and replicated from 6 to 11 times. Blocks of treatments were separated by 30-foot border strips to lessen contamination between insecticides by drift. The insecticides were applied with a truck-mounted, low-pressure, low-gallonage "Yellow Devil" weed sprayer using 20 gallons of spray per acre at 40 p.s.i. of pressure from an 18-foot, brush-type boom with 11 No. 8002 T-jet nozzles.

Plant bug counts were taken at various intervals by taking 25 net sweeps per plot. Seed yields were estimated by counting the number of legumes in several square-foot areas that were selected systematically. This technique for estimating seed yields was developed as a part of the studies reported here and will be described briefly along with factors influencing its development.

If seed yields are taken in a conventional manner, the trefoil has to be mowed, then dried before threshing; during this drying period much of the seed may be lost through shattering of the legumes. The mowing of trefoil, drying of the foliage (if it is to be dried where shattered seed may be collected), threshing the legumes, and cleaning the seed require a great deal of time, space, and equipment. The task becomes increasingly difficult if the

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[2] The authors are indebted to S. W. Jacklin for aiding in the field work and to H. H. Shorey for suggestions concerning experimental design.

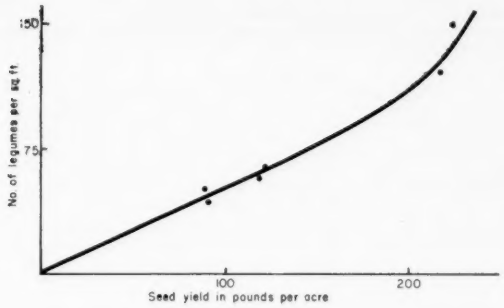

FIG. 2.—The relationship between the number of legumes per square foot and the estimated seed yield in pounds per acre.

experimental areas are a great distance apart, and experimentation is conducted on privately owned farms. In view of these circumstances it seemed worthwhile to develop a method for estimating seed yields to aid in evaluating insect control.

Past experience has shown that 20- by 50-foot plots are convenient and practical units with which to work. With this plot size in mind a sampling frame was constructed, which could be used to sample a 10- by 40-foot area. This area would be equivalent to a 20- by 50-foot plot minus a 5-foot border strip on all sides. Strings were attached to the sides and ends of the sampling frame at 1-foot intervals, thus a "cross-hatching" of strings formed square foot areas. Using this sampling frame all of the legumes in each square foot of six 10- by 40-foot areas were counted. In each case, after the legumes had been counted, the crop was mowed, threshed, and the seed cleaned and weighed. The actual seed yields are plotted against the mean number of legumes per square foot in figure 2.

For each of the six replicates of 400 square-foot legume counts, the variance and the standard deviation of the mean expressed as a percentage of the mean for eight different sample sizes were calculated. An example of one of the variance curves which was drawn after plotting the standard deviation of the mean expressed as a percentage of the mean against the sample size is shown in figure 3. The optimum sample size seems to lie between 12 and 24 square feet for a 10- by 40-foot area, because this range falls beyond the points of maximum curvature of the variance curve. From this information a sample size of 20 square feet per plot was selected arbitrarily.

From the eye-fitted line in figure 2 it is possible to estimate seed yields in pounds per acre from the number of legumes per square foot. It should be noted that as the number of legumes becomes more numerous the relationship is no longer linear, since the number of seeds per legume decreases as the legume density increases.

All statistical analyses were conducted using the actual legume counts, but the conversion of the number of legumes into pounds per acre makes it possible to obtain a more practical evaluation of the results. Tukey's h.s.d. test was used to test for differences between means in all the experiments with the exception of one in which a factorial arrangement was used. Here, the F test was applied. All testing was done at the 5% level.

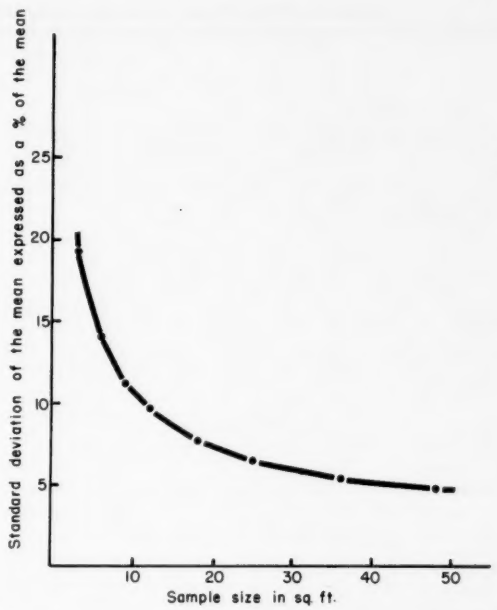

FIG. 3.—The standard deviation of the mean expressed as a percentage of the mean plotted against the sample size in number of square feet.

RESULTS.—*Experiment I.*—In an experiment laid out in a factorial arrangement, endrin was applied at 0.125 pound per acre for control of the meadow spittlebug, *Philaenus leucophthalmus* (L.) on May 10, 1959, and aldrin was applied at 1.0 pound per acre for plant bug control of June 10, 1959. Bug counts from net sweepings were taken 2 and 3 weeks after the aldrin application. The results of the statistical analysis of the legume counts which were taken on August 3, 1959 are presented in table 1.

For easy comparison the pertinent data are summarized in table 2 along with estimated seed yields in pounds per acre.

Endrin, applied to control the meadow spittlebug, failed to increase seed yields, but the aldrin, applied to control the tarnished plant bug, resulted in significant increases in seed yields. The data on insect counts given

Table 1.—Results of statistical analysis of legume counts from a control experiment on birdsfoot trefoil grown for seed. Aurora, New York. 1959.

Analysis of Variance				
Source of Variation	d.f.	S.S.	M.S.	F
Treatments	3	7,883.2	2,627.7	9.74[a]
Endrin vs. no aldrin	1	167.0	167.0	0.62
Aldrin vs. no endrin	1	7,707.8	7,707.8	28.58[a]
Interaction	1	8.4	8.4	0.03
Replicates	5	1,835.6	367.1	1.36
Error	15	4,044.8	269.7	—

[a] Significant at the 5% level.

Table 2.—Effect of insecticides applied to birdsfoot trefoil to control the tarnished plant bug.

Treatment	Pounds of Actual Toxicant per Acre	No. of Insects[a] After Application				No. of Legumes per Sq. Ft.	Estimated Seed Yield (lb./a.)
		2 Weeks		3 Weeks			
		Tarnished Plant Bug	Other Mirids	Tarnished Plant Bug	Other Mirids		
		A. *Aurora, New York, 1959*					
Aldrin and endrin	1.0 0.125	2.8	3.5	11.7	14.5	140.5	220
Aldrin	1.0	3.1	2.6	12.5	10.8	134.1	215
Endrin	0.125	29.3	9.0	26.8	10.5	103.5	190
Untreated	—	36.0	17.8	33.7	15.3	99.4	185
		B. *Delanson, New York, 1959*[b]					
DDT	1.0	—	—	3.3	15.1	155.9	235
Aldrin	1.0	—	—	1.8	21.7	150.9	230
Toxaphene	2.0	—	—	3.1	16.3	141.6	220
Untreated	—	—	—	6.1	27.1	131.9	210
		C. *Westport, New York, 1959*[b]					
Aldrin	1.0	0.0	3.3	9.7	8.2	71.4	135
DDT	1.0	0.4	3.9	12.7	10.2	65.3	120
Toxaphene	2.0	1.3	3.8	12.4	14.7	62.7	115
Untreated	—	14.7	7.3	31.0	16.1	48.9	95
		D. *Delanson, New York, 1958 and 1959*[b,c]					
Parathion	1.0	—	—	3.4	17.6	101.1	190
Aldrin	1.0	—	—	3.2	24.0	98.7	185
Endrin	0.25	—	—	5.4	21.2	88.3	160
Malathion	1.0	—	—	5.4	20.4	82.3	155
Untreated	—	—	—	33.2	29.0	70.2	135

[a] Average number of insects taken in 25 net sweeps per plot.
[b] All means flanked by the same line are not significantly different according to Tukey's h.s.d. test at the 5% level.
[c] These counts are from five replicates applied in 1959.

in table 2,A indicate an evident relation between the number of mirids present, particularly the tarnished plant bug, and seed yields.

Experiment II.—Aldrin and DDT were applied at 1.0 pound per acre and toxaphene was applied at 2.0 pounds per acre to Empire birdsfoot trefoil on June 11, 1959. Insect counts were made 3 weeks after application, and legume counts were taken on July 28, 1959 (table 2, B). The mean numbers of legumes from the treated plots were somewhat larger than those from the untreated plots, but they were not significantly greater. This lack of significance may be explained by the rather low insect counts in the untreated plots.

Experiment III.—Insecticide treatments, which were identical to those in experiment II, were applied to Empire birdsfoot trefoil on June 12, 1959 at Westport, N. Y. Insect counts from net sweeps were taken 2 and 3 weeks after application, and legume counts were taken on July 28, 1959 (table 2,C). Good control was obtained with all insecticides, and all materials resulted in significant increases in seed yields over the untreated checks. Table 2,C also shows very clearly that low insect counts in the treated plots corresponded to the higher seed yields.

Experiment IV.—Aldrin, parathion, and malathion at 1.0 pound per acre and endrin at 0.25 pound per acre were applied to six replicates of Empire birdsfoot trefoil on June 17, 1958 and to five replicates on June 11, 1959 at Delanson, N. Y. In 1959 legume counts were taken on July 28; the 1958 results have been reported previously by Ridgway & Gyrisco (1959). The data from the 1958 and 1959 experiments (summarized in table 2,D) were quite uniform and were pooled for broader inferences and for the purpose of making recommendations on a state-wide basis. Although all materials gave reasonably good insect control, only parathion and aldrin increased seed yields significantly. These two materials also gave significant increases in seed yields over plots treated with malathion. Here again, particularly with the tarnished plant bug, good control corresponded with greater seed yields.

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Forceps Design for Transferring Honey Bee Eggs[1]

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ABSTRACT

Forceps modified from ordinary dissecting forceps can be used successfully to move eggs of any age of the honey bee (*Apis mellifera* L.). Two factors are critical in the design. The opening at the tips should be 0.23 mm., and the underside of the forceps at the tips must be flared to prevent contact with the posterior sticky end of the egg. About 75% hatch of the eggs transferred may be expected whether they are developed inside the hive or in the incubator. The forceps are illustrated by photographs.

Transfer or "grafting" 12- to 24-hour larvae of honey bees (*Apis mellifera* L.) has been practiced for many years in commercial queen production. A special forceps has been designed which now permits successful transfer of the eggs from their cells to new locations without injury.

Recent literature on the moving of bee eggs by DuPraw (1960), Shuel & Dixon (1959), and Smith (1959) describes the removal of a small piece of the wax comb surrounding the point of attachment of the egg. A tool has also been invented in Germany to punch out the base of the worker cell containing the egg.

Moving bee eggs may be useful for several reasons: (1) Queens can be reared from eggs either commercially or experimentally; (2) newly hatched larvae are available for laboratory work; and (3) the egg itself can be studied under controlled conditions. This paper describes a forceps modified from an ordinary dissecting forceps which can be used successfully to move eggs of any age.

DESCRIPTION.—Two factors are critical for the successful design of the forceps: (1) The opening between the two prongs for grasping the egg should be 0.23 mm. (0.009 inch); (2) the underside of the forceps tip—the portion that will come close or touch the bottom of the cell where the egg is attached—must be flared (fig. 1A, B and C, bottom views) so that the part of the egg in contact with the cell is not touched by the tool. The other shaped parts may vary to satisfy individual preference.

The two forceps arms are treated as mirror images. The width of each arm is tapered from 2 to 1 mm. at the terminal 2.5 cm., while the thickness is tapered from 1 to 0.5 mm. over the same length (fig. 2, part A). The arms are bent at a point 2.3 to 2.7 cm. from the tips, at an angle of about 45°. This bend enables the operator to insert the forceps into the cells parallel to their sides without obstructing his view of the egg and the forceps tips. After the two arms are made nearly identical, the tip is bent 90° in the opposite direction at a point 2 to 2.5 mm. from its end; its upper side then measures 1 to 1.5 mm. from tip to bend (fig. 2, part B).

After alignment of the two tips the portion that will grasp the egg may either be bent out or ground to 0.23 mm. (fig. 2, part C).

Tools used for grinding and polishing are small, fine-cut, flat and round files and oil stones. Very fine emery paper is adequate for polishing.

Tension on the forceps should be very slight. Separation of the tips up to the first bend should be such that they easily enter the cell for its entire depth without touching the sides. The width of the forceps plus the opening between the tips is 3 mm. where the points come together and 5 mm. at the 45° bend before pressure is applied.

Use of the forceps has demonstrated that there is considerable difference in egg thickness, as discussed by DuPraw (1960), and about one-third of the eggs slip through the 0.23 mm. opening if the operator attempts to move the egg straight up and out of the cell. However, almost all upright eggs may be transferred if the operator will move the forceps slightly sidewise before lifting from the cell. After these eggs have been removed, they are placed upright on the desired surface with the same sidewise motion.

Eggs occasionally will stick to the forceps instead of to the new surface. When this happens, the forceps must be cleaned by gentle rubbing with a soft cloth.

EXPERIMENTAL RESULTS.—Data from two tests are presented to show the approximate success to be expected in moving eggs.[3] The first test was performed with eggs removed from the cells and placed in an incubator maintained at 35° C. and at a relative humidity of approximately 90%. The second test was performed with eggs placed in queen cell-cups in attempts to rear queens from them under different conditions.

In the first test, bee eggs were placed on three different surfaces in an upright position much the same as if a queen had deposited them. Surfaces were filter paper, aluminum foil, and pieces of beeswax foundation. Success was measured by the resulting hatch. Eggs placed on filter paper did not hatch well in several experiments because of the presence of mold and bacteria. No foreign growth was observed on eggs placed on beeswax foundation or aluminum foil. The results obtained are summarized in table 1.

Eggs moved into the incubator came from three differ-

Table 1.—Incubator hatch of honey bee eggs placed, in the normal position, on different surfaces.

Surface	No. of Eggs Moved	No. of Eggs Hatched After			Per Cent Hatch
		1 Day	2 Days	3 Days	
Comb foundation	108	17	15	71	95
	137	70	21	0	66
	900	124	303	271	78
	800	464	118	166	93
Aluminum foil	200	53	50	41	72
Filter paper	300	82	47	14	48
Total	2,445	810	554	563	79

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[2] In cooperation with Louisiana State University.
[3] Zofia Konopacka, of Skierniewice, Poland, gave advice and assistance in moving some of the eggs in these experiments.

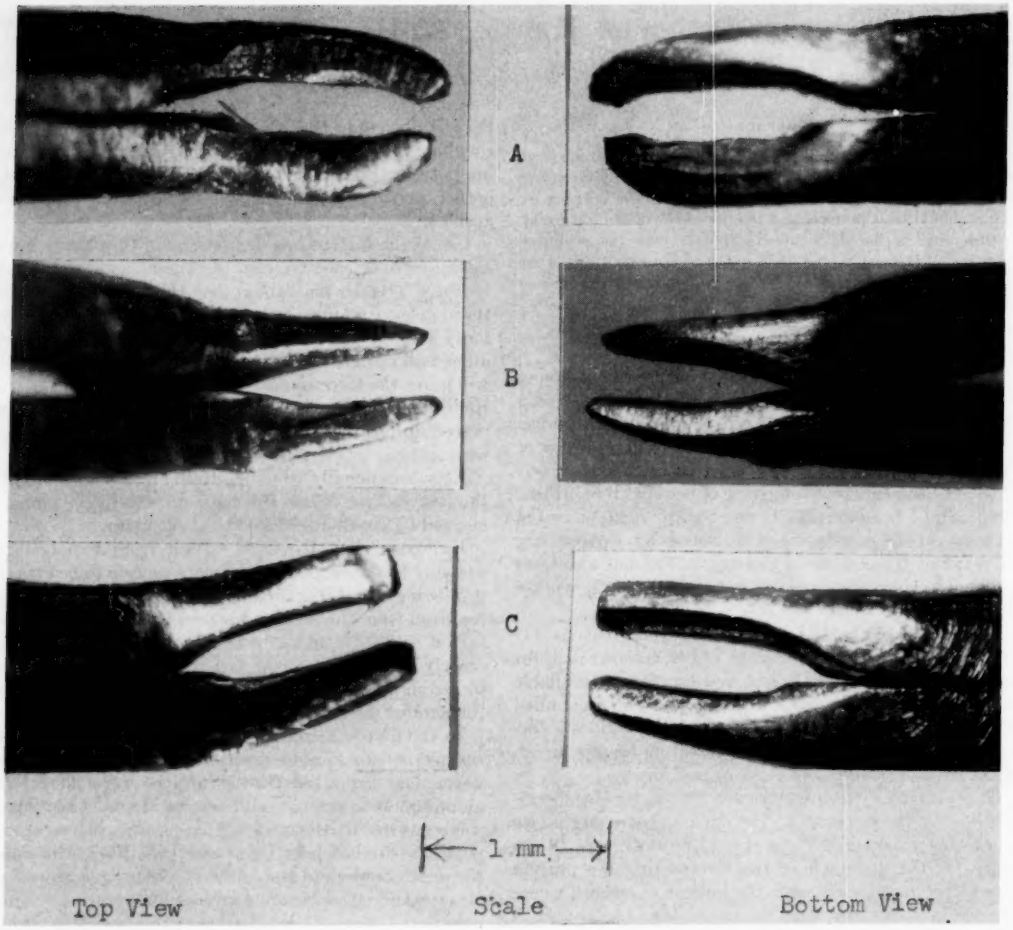

FIG. 1.—Three different designs of egg transfer forceps tips showing opening of 0.23 mm. and flaring on bottom necessary to avoid sticky posterior part of egg. Photographs by J. H. Roberts.

ent queens and were of all ages. No special significance was attached to the large first-day hatch. Probably more older eggs were picked up, which resulted in this grouping bias. Moving eggs with the forceps is a relatively simple procedure, as is evidenced by the fact that the sample of 900 eggs (table 1) was transferred in 3½ hours without the aid of magnification. (fig. 3)

In the second test, rearing queens directly from transposed eggs instead of young larvae was tried. Of 1,750 eggs moved into queen cell-cups, 1,232 (70%) were accepted. The following generalizations are made of the many different transfer procedures tested:

(1) Eggs and larvae placed with 3 to 4 pounds of queenless, broodless, and enclosed bees for 24 hours.
(2) Eggs only placed in swarm box for 24 and 36 hours.
(3) Eggs placed in a queenless, broodless colony for 24 hours.
(4) Eggs placed above a queen excluder in a colony with a queen ("finishing" or cell-building colony) among frames of brood for 24 hours.
(5) Eggs placed in a queen-rearing colony, as defined by Hoopingarner (1959). This colony contains a queen confined to several frames with eggs only, no young brood, a large amount of sealed brood (older and about to emerge), and young bees.

Acceptance was good under all these conditions provided eggs were placed in queen cell-cups polished by the bees.

DISCUSSION.—There has been a long-standing dream among bee men to raise queens from eggs because of the possibility of rearing superior queens, or queens that can lay more eggs. Very little data support the idea that a queen raised from an egg is better than one raised from a larva. Genetically, queens and workers have the same inheritance; the difference is caused by environment. Data from Shuel & Dixon (1959) on larvae less than 30 hours old, destined to be either workers or queens, showed a difference in the food, or carbon dioxide evolved by the food, on which the larvae were feeding.

Haydak (1943) has shown that there is no differentia-

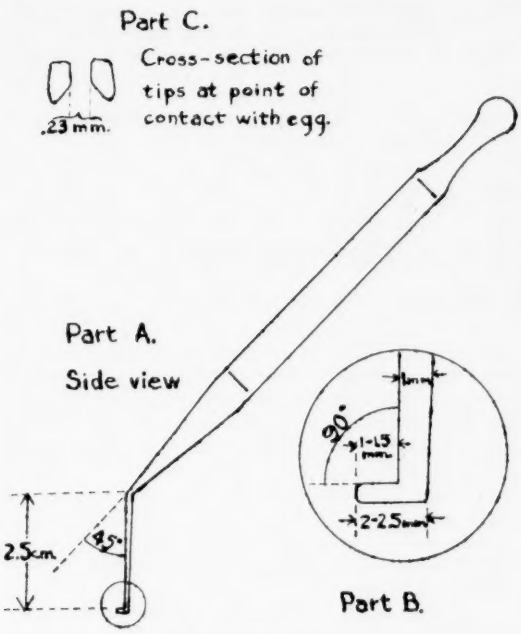

FIG. 2.—Design of forceps.

tion as to development in larvae held in either worker or queen cells for the first 48 hours. Smith (1959) has reared workers, queens, and intermediate forms in the incubator all in the same dish and on the same food.

In the experiments reported here, very good queens, poor queens, and even worker bees have been reared from eggs in queen-cups, all at the same time and in the same colony. This extreme variation in development does not usually occur when rearing queens from young larvae. A statement, *per se*, that queens reared directly from eggs are superior is not yet justified. A method for rearing queens from eggs is presently being investigated.

The free shipment and importation of adult honey bees is now restricted in the United States and other countries to prevent the spread of the internal mite, *Acarapis woodi* (Rennie). Bee semen can now be stored for several months and sent to any place in the world (Taber & Blum 1960). Unfortunately, this process allows only half of the genetic stock to be imported. If a method could be developed to ship and store eggs as easily as semen, there would be no difficulty in sending breeding stock to any place desired. The author believes that the forceps described will assist in the development of a way to safely store honey bee eggs for shipment.

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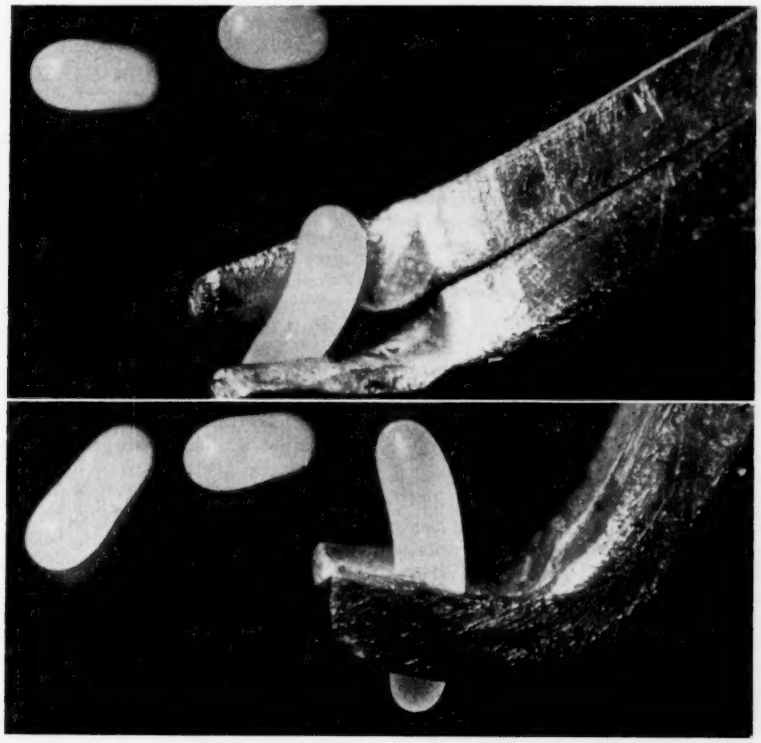

FIG. 3.—Forceps with eggs of the honey bee. Photographs by J. H. Roberts.

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Distribution of Army Cutworm Larvae in Wheat and Barley Fields[1]

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ABSTRACT

Populations of the army cutworm, *Chorizagrotis auxiliaris* (Grote), tended to be higher in winter barley than winter wheat planted side by side in the same field. Highest populations occurred in barley within 30 feet of the adjoining wheat. Distribution of larvae in linear 1-foot samples agreed closely with the Poisson. Practical application of these results are discussed.

The army cutworm, *Chorizagrotis auxiliaris* (Grote), is an important pest of wheat, barley, alfalfa and other crops in Nebraska. Winter barley, because of wheat acreage allotments, is becoming of increasing importance in western Nebraska and has been especially susceptible to cutworm injury. Whether this susceptibility to injury might be caused by higher cutworm populations, as reported by farmers, seemed worthy of investigation.

METHODS.—Six comparisons of larval populations were made in 1959 using paired winter wheat and barley fields on the same farms in Keith County. Fields were selected which had received similar cultural practices and were seeded about the same time. In no case was there any barrier between the paired fields with a maximum of 2 feet of cultivated ground separating the two. Sampling was begun 100 feet into each field from the adjoining road and continued at 10-foot intervals for another 90 feet parallel to the common boundaries. Paired samples were taken at distances of 3, 10, 20, 30, 50 and 100 feet into each crop perpendicular to the common boundary. One linear foot of soil was examined on each side of the row nearest these measured distances. The width of these sample units varied from 8 to 12 inches, depending on row spacing, but always included the entire distance to the next row. Row spacing was the same in each pair of fields sampled and all rows ran parallel to the common border. This sampling procedure, while not allowing actual comparisons of populations between different pairs of fields without adjusting for sample width, clearly defined differences between wheat and barley in each pair of fields. Linear-foot counts were transformed by $\sqrt{x+1}$ prior to analysis and Duncan's (1955) Multiple Range Test was used to make comparisons.

RESULTS.—Comparisons of populations at different distances into paired wheat and barley fields from the common boundary are given in table 1. These results are presented graphically in figure 1 in terms of actual populations rather than transformed values.

Significantly higher populations were found in barley than in wheat in five of the six paired fields. In Field 6 wheat had slightly, but not significantly, more cutworms than barley. Greatest fluctuations in cutworm populations occurred within 30 feet of the common boundary. In no case was there a significant difference between samples taken at 50 and 100 feet into either crop from this common border and counts made at these distances likely were more representative of the field as a whole. Using only samples taken at 50 and 100 feet as a basis for comparing the two crops, barley was still found to have significantly higher populations in four fields with no difference in two.

Samples taken across wheat and barley parallel to the common boundary showed significant differences to exist in that direction only in Field 3. In this field there were more cutworms in both wheat and barley in the first 50 feet nearer the road. Heavier marginal infestations have been observed to occur commonly in both crops. Sampling areas in this study were purposely located 100 feet away from adjoining roads to avoid such variation, leaving only the effect of the wheat-barley boundary to explain.

Although varying in distance and intensity, the most notable feature revealed by these samples was a peak population in barley within 30 feet of the common boundary. This effect was noted in five of the six fields. Also in all but Field 4 this peak was accompanied by lower counts in wheat within 20 feet of the boundary. These peaks and depressions always fell within about 30 feet of each other. It would appear that winter barley, under identical cultural practices, had higher cutworm populations than wheat and that the factor responsible was operating most strongly along the boundary between the two crops. This effect was most marked in fields that had lower populations. Although the reasons for this larval distribution are unknown, certain practical uses may be made of this information without knowlege of its causes.

PRACTICAL APPLICATION OF RESULTS.—When making surveys for the army cutworm it would appear highly desirable that samples be taken 50 to 100 feet or more away from any road or adjoining field. As shown, samples taken within 30 feet of the field edge may give highly misleading estimates of the population occurring over the field as a whole. Stratified random samples, when economically feasible, would be even more appropriate. Such a sampling procedure should certainly be used when de-

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Table 1.—Mean number of army cutworm larvae per linear foot of row at different distances from the common boundary in six paired wheat and barley fields.[a]

Feet into Field	Field[b] 1	2	3	4	5	6
			Wheat			
100	1.27 cdef	1.28 abc	1.14 de	1.46 e	1.39 cd	1.52 abc
50	1.21 def	1.18 bcd	1.18 cd	1.59 bcde	1.40 cd	1.55 abc
30	1.16 efg	1.12 cd	1.18 cd	1.52 de	1.63 abc	1.69 ab
20	1.09 fg	1.12 cd	1.12 de	1.62 bcde	1.61 bc	1.79 a
10	1.04 g	1.20 bcd	1.02 f	1.55 cde	1.71 ab	1.38 cd
3	1.04 g	1.08 d	1.06 ef	1.75 abcd	1.31 d	1.48 bcd
			Barley			
3	1.35 bcd	1.39 a	1.53	1.67 bcde	1.55 bcd	1.60 abc
10	1.40 abc	1.35 ab	1.32 ab	1.76 abcd	1.64 abc	1.65 abc
20	1.52 a	1.38 a	1.36 a	1.84 ab	1.78 ab	1.46 bcd
30	1.29 bcde	1.27 abc	1.28 ab	1.96 a	1.89 a	1.54 abc
50	1.32 bcde	1.31 ab	1.26 bc	1.82 abc	1.62 bc	1.49 bcd
100	1.44 ab	1.29 abc	1.32 ab	1.79 abcd	1.54 bcd	1.22 d

[a] Data transformed by $\sqrt{x+1}$.
[b] Any two means in the same column not followed by the same letter are significantly different at the 5% level.

termining the need for control measures. It seems likely that in many cases only the more heavily infested border of a field would warrant treatment.

In research work, the design of the experiment should take into consideration these marginal changes in population. If uniform infestations are desired, location of plots as near the center of a field as possible would be indicated. If field margins are to be used for convenience or to obtain higher infestations, it would seem advisable to place blocks of plots parallel to these margins. Field corners, in which variation would be expected to occur in two directions, should be avoided if possible or a design such as a latin square used which will permit easy removal of both row and column effects.

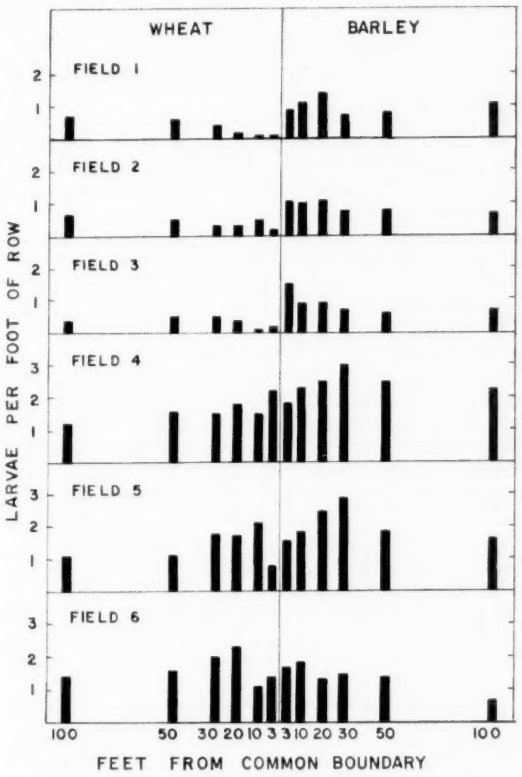

FIG. 1—Army cutworm populations in six paired wheat-barley fields at different distances from the common boundaries.

MATHEMATICAL DISTRIBUTION OF LARVAE.—Larval counts in linear 1-foot samples in all fields studied agreed closely with the Poisson distribution. Table 2 gives the observed and theoretical distributions for one pair of fields. Counts in other fields fitted the Poisson equally well.

A square root transformation suggested by Snedecor (1956) for low counts agreeing with the Poisson was used in this study to gain precision. However, when several samples are pooled and totals analyzed, as is commonly done in chemical control studies, the use of a transformation will not ordinarily lead to conclusions any different from those reached by an analysis of the original data.

Table 2.—Frequency distribution of army cutworm larvae in linear 1-foot samples and comparison with Poisson distribution.

Larvae per Foot	Field II—Wheat Observed Frequency	Field II—Wheat Expected Frequency	Field II—Barley Observed Frequency	Field II—Barley Expected Frequency
0	28	29.1	17	16.7
1	40	41.2	37	32.9
2	33	29.2	29	32.5
3	14	13.8	20	21.4
4	3	4.9	8	10.6
5	2	1.4	5	4.2
6+	0	0.4	4	1.7
Total	120	120.0	120	120.0
	$\chi^2 = 0.99$		$\chi^2 = 3.26$	
	$P = >0.90$		$P = >0.65$	

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A Comparison of Dimethoate Administered as an Injection and in Supplemental Feed for Control of Cattle Grubs[1]

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ABSTRACT

Hereford calves from Sweet Grass County, Montana were treated late in January with dimethoate for control of larvae of cattle grubs (*Hypoderma* spp.). The compound was administered either as a single injection at 10 mg./kg. or for 10 days in a supplemental feed at 2 mg./kg./day. Intramuscular injection resulted in 81% control and low-level feeding in 79% control of cattle grubs. Of calves receiving dimethoate in the feed or as an intramuscular injection, 80% and 84%, respectively, had fewer than six grubs; however, 56% and 20%, respectively, were completely free of grubs. Of 25 calves receiving dimethoate in the feed only three harbored 60% of the grubs. It would therefore appear that some calves failed to take the supplemental feed, but in those which did, control was good.

Reported herein are the results of a single experiment in which the efficacy of dimethoate (Amer. Cyanamid 12,880; *O,O*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorodithioate) was studied when administered to calves for control of cattle grubs, *Hypoderma lineatum* (DeVill.) and *H. bovis* (L.). The efficacy of a single intramuscular injection at 10 mg./kg. and feeding of 2 mg./kg./day for 10 days were compared. Since it appeared that dimethoate was most effective against the larvae after they reached the back (Hewitt *et al.* 1958), treatment was delayed until some larvae reached the back, but before any damage to the hide had occurred. Previous experience with calves from the same area had shown that only negligible damage to the hides occurred before February. Administration of dimethoate was therefore instituted during the latter half of January.

METHODS.—Seventy-five calves were purchased for this experiment from a single ranch in Sweet Grass County, Montana. The calves were trucked to the Huidekoper Veterinary Research Station at Big Timber, Montana, on November 9, 1959. At this time they ranged in age from 6 to 8 months and weighed an average of 389 pounds. The calves were maintained in corrals at the Station for the duration of the experiment and fed a grass-type hay.

On January 12, 1960, the calves were allotted at random to three groups of 25 each and then weighed as groups. On this date the backs of several calves were examined for the presence of grubs but none were found. Beginning January 13 and continuing through January 30, all the calves were fed free choice 1 pound per head per day of a supplemental meal containing 32% protein. From January 21 through January 30, a 10-day period, the calves in one group received dimethoate in the supplemental feed at a dosage of 2 mg./kg. of body weight/day.

Table 1.—Cattle grub control and weight gains in calves receiving dimethoate either by intramuscular injection or in the feed.

CATEGORY	DIMETHOATE ADMINISTERED		
	10 Days in Feed (2 mg./kg./day)	Intramuscular Injection (10 mg./kg.)	None
No. of calves	25	25	25
Peak average grub counts	3.1	2.8	14.9
Control (%)	79	81	—
No. with less than 6 grubs	20 (80%)	21 (84%)	5 (20%)
No. without grubs	14 (56%)	5 (20%)	1 (4%)
Average gain Jan. 12 to April 27 (lb.)	77	80	81

On January 25, the calves in the second group were injected intramuscularly with dimethoate at a dosage of 10 mg./kg. The third group remained as untreated controls.

Counts of the number of grubs appearing in the backs were made by palpation on February 2, March 1, April 1 and April 27.

RESULTS.—In the untreated calves, the count on February 2 revealed an average of 4.4 grubs per calf; on March 1 and April 1 the average count was 9.8. The average count declined slightly to 9.0 at the termination of the experiment on April 27 (fig. 1). Of the treated calves, those receiving dimethoate in the feed had an average of 0.8, 1.5, 1.5, and 1.6 grubs per calf on February 2, March 1, April 1, and April 27, respectively. The calves which were given dimethoate by injection had an average of 1.4, 0.9, 1.6, and 1.6 grubs per calf on the four respective counting dates.

Peak average grub counts for each group (table 1) were obtained by averaging the highest number of grubs found in each calf of the group during the period of observation. These figures were used for determining the percentage reduction in grub populations in the treated groups as compared with the untreated group. Percentage control was approximately the same in each of the treated groups, being 79% in the group receiving 2 mg./kg./day of dimethoate in the feed for 10 days, and 81% in the group receiving dimethoate as an intramuscular injection.

There were no appreciable differences in gain of weight among the groups during the experiment. No intoxication was observed in any of the calves following adminis-

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[2] Associate Parasitologist and Assistant Veterinarian, respectively. The assistance of Mr. Walter Braughton in the care and handling of the experimental animals is gratefully acknowledged.

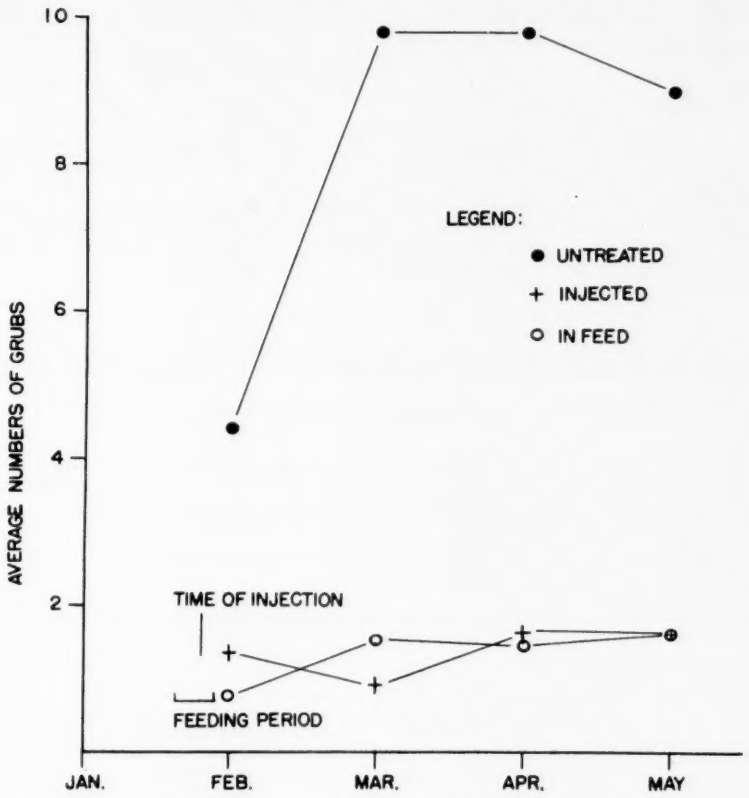

FIG. 1.—Average number of cattle grubs in calves treated with dimethoate by intramuscular injection or by administration in the feed, and in untreated calves.

tration of dimethoate either by injection or in the feed at the dosage levels used.

DISCUSSION.—At safe dosage levels, dimethoate has shown considerable variation in its action against the larvae of *Hypoderma*. Hewitt *et al.* (1958) observed complete kill of the second instar and 67% to 100% kill of the third instar when dimethoate was administered orally. In several tests carried out by various workers, control ranged from 20% to 79% when dimethoate was administered by mouth at 10 mg./kg. (Howell *et al.* 1958, Defoliart *et al.* 1958, Neel 1958). Rogoff *et al.* (1960) reported 94% control of grubs at 15 mg./kg. When dimethoate was injected intramuscularly, Hewitt *et al.* (1958) reported 100% kill of the third instar, 86% to 100% kill of the second instar and 41% to 54% kill of the first instar at a dosage level of 10 mg./kg. Neel (1958) observed 58% control and Drummond (1959) observed 51% to 92% control of grubs at 10 mg./kg. given intramuscularly. At a somewhat higher dosage level, 15 mg./kg., Rogoff *et al.* (1960) observed 94% control. The control which we observed when dimethoate was either injected once or fed for a 10-day period, 81% and 79% control, respectively, is representative of what others have reported.

Although in this experiment there was no significant difference between the average control obtained by the two methods of administration, nearly three times more calves in the fed group were completely free of grubs than in the injected group (table 1). In the group that received dimethoate in the feed, three calves had peak counts of 17, 19 and 10, comprising 60% of the grubs in the 25 calves. It is likely that these calves failed to take the medicated feed. No other calves had peak counts higher than six. In the injected group, on the other hand, five calves were completely free of grubs, but only one had more than seven grubs. Complete control occurred in only a few calves but complete failure was also rare.

A short time after treatment, the numbers of grubs palpable in the backs of the treated calves were considerably below those in the untreated group and numbers in the treated groups remained at the same average level throughout the observation period (fig. 1). This permits the inference that dimethoate was as effective against larvae which had not yet reached the back as it was against larvae present in the backs at the time of treatment. Also, the compound was apparently equally effective against *H. lineatum* and *H. bovis*, both of which occur in this area. A rise in numbers in the treated groups late in the experimental period would have indicated a lower efficacy of dimethoate against larvae not yet present in the backs, against *H. bovis* which appears later than *H.*

lineatum, or both. Late treatment did not, however, give complete grub control at the dosages used in this experiment.

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Pesticide Names Found in U.S.S.R. Technical Literature

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There has recently been a great increase in interest in Soviet scientific literature on the part of American research workers. It is believed that the following list of pesticide names occurring in U.S.S.R. books and journals dealing with agricultural chemicals and entomology will be useful to those who have occasion to consult either the original Russian literature or translations and abstracts from it. The list is arranged alphabetically according to the names as transliterated in the Latin alphabet. The names are also shown in their original form in the Cyrillic alphabet and their English equivalents or definitions are given.

RUSSIAN NAME		ENGLISH NAME OR DEFINITION
Latin Alphabet	Cyrillic Alphabet	
al'bikhtolovaya pasta	альбихтоловая паста	albichthol paste; an oily liquid prepared from shale; 2% emulsion
aldrin	алдрин	aldrin
allilkhlorid	аллилхлорид	allyl chloride
anabadust	анабадуст	anabasine sulfate dust
anabasin-sul'fat	анабасин-сульфат	anabasine sulfate
arsenat kal'tsiya	арсенат кальция	calcium arsenate
arsenit kal'tsiya	арсенит кальция	calcium arsenite
arsenit natriya	арсенит натрия	sodium arsenite
bakovaya smes'	баковая смесь	tank mix of oil, water, and blood albumin
barii khloristyi	барий хлористый	barium chloride
barii uglekislyi	барий углекислый	barium carbonate
betanaftol	бетанафтол	2-naphthol
bismetan	биэметан	bis(*p*-chlorophenoxy)methane
bladan	бладан	TEPP
bordoskaya zhidkost'	бордоская жидкость	Bordeaux mixture
bromistyi étil	бромистый этил	ethyl bromide
bromistyi metil	бромистый метил	methyl bromide
DDD	ДДД	TDE
DD-smes'	ДД-смесь	DD-mixture (1,3-dichloropropene + 1,2-dichloropropane)
DDT	ДДТ	DDT
derris	деррис	derris
detoil	детоил	DDT, chlorobenzene, spindle oil, sulfonated fish oil, and ammonium naphthenate soaps—an emulsion concentrate
diazinon	диазинон	Diazinon(*O,O*-diethyl *S* (and *O*)-2-(ethylthio) ethyl phosphorothioate)
diéldrin	диэлдрин	dieldrin
dikhloral'mochevina	дихлоральмочевина	1,3-bis(2,2,2-trichloro-1-hydroxyethyl)urea
dikhloretan	дихлорэтан	1,2-dichloroethane, ethylene dichloride
dinitro-o-krezol	динитро-о-крезол	4,6-dinitro-*o*-cresol
dinitro-o-vtor-butilfenol	динитро-о-втор-бутилфенол	2-*sec*-butyl-4,6-dinitrophenol
dinitrorodanbenzol	динитророданбензол	2,4-dinitrophenyl thiocyanate
ditio	дитио	sulfotepp
ditiofos	дитиофос	sulfotepp
DKhÉ	ДХЭ	1,2-dichloroethane; ethylene dichloride
éfirsul'fonat	эфирсульфонат	ovex
éndotal	эндотал	disodium salt of 7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid

RUSSIAN NAME		ENGLISH NAME OR DEFINITION
Latin Alphabet	Cyrillic Alphabet	
étil bromistyi	этил бромистый	ethyl bromide
étilksantogenat natriya	этилксантогенат натрия	sodium ethylxanthate
figon	фигон	dichlone (Phygon)
flitsid	флицид	a perfumed pyrethrum extract used against house flies and mosquitoes
formalin	формалин	formalin; formaldehyde 40% w/w in water
fosfid tsinka	фосфид цинка	zinc phosphide
ftoralyuminat natriya	фторалюминат натрия	sodium fluoaluminate, cryolite
ftoristyi natrii	фтористый натрий	sodium fluoride
fuklazin	фуклазин	20% wettable powder of ferric dimethyldithiocarbamate
geksakhloran	гексахлоран	BHC
geksakhlorbenzol	гексахлорбензол	hexachlorobenzene
geksakhlortsiklogeksan	гексахлорциклогексан	BHC
geksaten	гексатен	mixture of chlorinated terpenes and isomers of BHC
geksaétiltetrafosfat	гексаэтилтетрафосфат	hexaethyl tetraphosphate
geptakhlor	гептахлор	heptachlor
gezarol	гезарол	Gesarol (DDT formulation)
dizel'noe toplivo	дизельное топливо	diesel oil
GKhTsG	ГХЦГ	BHC
gusenichnyi klei	гусеничный клей	"caterpillar glue," a thick, sticky preparation of resins, turpentine, rosin, and coal tar mixed with oils
ISO	ИСО	lime-sulfur
izodrin	изодрин	isodrin
izopestoks	изопестокс	*N,N*-diisopropylphosphorodiamidic fluoride
izopropil-3-khlorfenilkarbamat	изопропил-з-хлорфенилкарбамат	*N*-3-chlorophenylisopropylcarbamate
izopropilfenilkarbamat	изопропилфенилкарбамат	*N*-phenylisopropylcarbamate
izvest' gashenaya	известь гашеная	slaked lime
izvest' khlornaya	известь хлорная	chloride of lime
izvest' negashenaya	известь негашеная	quick lime
izvestkovo-sernyi otvar (ISO)	известково-серный отвар	lime-sulfur
kal'tsii mysh'yakovistokislyi	кальций мышьяковистокислый	calcium arsenite
kal'tsii mysh'yakovokislyi	кальций мышьяковокислый	calcium arsenate
kal'tsinirovannaya soda	кальцинированная сода	soda ash, sodium carbonate
kaptan	каптан	captan
karbofos	карбофос	malathion
karbolineum	карболинеум	Carbolineum (chlorinated anthracene oil)
kerosin	керосин	kerosine
khlorindan	хлориндан	chlordane
khloristyi barii	хлористый барий	barium chloride
khloristyi étilen	хлористый этилен	1,2-dichloroethane; ethylene dichloride
khlorokis' medi	хлорокись меди	copper oxychloride
khlorpikrin	хлорпикрин	chloropicrin
khlorsmes'	хлорсмесь	carbon bisulfide+carbon tetrachloride
khlorfen	хлорфен	chlorinated camphene containing 64–66% Cl
4-khlorfenildimetilmochevina	4-хлорфенилдиметилмочевина	3-(*p*-chlorophenyl)-1,1-dimethylurea
khlorfos	хлорфос	Dipterex(dimethyl 2,2,2-trichloro-1-hydroxyethyl phosphonate)
khlorten	хлортен	chlorinated pinene containing 67–69% Cl
kislota sinil'naya	кислота синильная	hydrocyanic acid
"Kontakt" Petrova	"контакт" Петрова	a sirupy liquid used as a wetting agent and containing sulfonic acids obtained from mineral oils
kremneftoristyi barii	кремнефтористый барий	barium fluosilicate
kremnoftoristyi natrii	кремнефтористый натрий	sodium fluosilicate
kreolin	креолин	Creolin (a coal-tar disinfectant, germicide, antiseptic, deodorant)
kriolit	криолит	sodium fluoaluminate, cryolite
kupfermeritol	купфермеритол	$Cu_2Ca(AsO_4)_2$; $CuCa_2(AsO_4)_2$
kuporos mednyi	купорос медный	cupric sulfate
kuporos zheleznyi	купорос железный	ferrous sulfate
kuzol-a	кузол-а	1-acetyl-1,2,3,4-tetrahydroquinoline
kuzol-f	кузол-ф	1-formyl-1,2,3,4-tetrahydroquinoline
KZM, KZMB, OR KZMV	КЗМ, КЗМБ, КЗМВ	an emulsion of "green" oil
margantsevokislyi kalii	марганцевокислый калий	potassium permanganate
masla mineral'nye	масла минеральные	mineral oils
mazut	мазут	thick, black liquid obtained on distillation of petroleum
mel	мел	chalk, calcium carbonate
merkaptofos	меркаптофос	demeton

RUSSIAN NAME		ENGLISH NAME OR DEFINITION
Latin Alphabet	Cyrillic Alphabet	
merkuran	меркуран	ethylmercuric chloride+γ-BHC
metafos	метафос	mixture of parathion and methyl parathion
metansul'foftorid	метансульфофторид	methylsulfonyl fluoride
metasistoks	метасистокс	β-mercaptoethyl dimethyl thiophosphate
metatsid	метацид	a mixture of "tiofos" and "metafos"
metil bromid	метил бромид	methyl bromide
metil bromistyi	метил бромистый	methyl bromide
metilformat	метилформат	methyl formate
metilsistoks	метилсистокс	β-mercaptoethyl dimethyl thiophosphate
metoksikhlor	метоксихлор	methoxychlor
mipafoks	мипафокс	*N,N'*-diisopropylphosphorodiamidic fluoride
myla tverdye	мыла твердые	hard soaps
myla zhidkie	мыда жидкие	soft soaps
naftalin	нафталин	naphthalene
naftol	нафтол	naphthol
natrii ftoristyi	натрий фтористый	sodium fluoride
natrii kremneftoristyi	натрий кремнефтористый	sodium fluosilicate
natrii mysh'yakovistokislyi	натрий мышьяковистокислый	sodium arsenite
natrii tsianistyi	натрий цианистый	sodium cyanide
natrii uglekislyi	натрий углекислый	sodium carbonate
nikotin	никотин	nicotine
nikotin-sul'fat	никотин-сульфат	nicotine sulfate
okis' etilena	окись этилена	ethylene oxide
oktakhlor	октахлор	chlordane
oktametil	октаметил	schradan
OP-7	ОП-7	polyethyleneglycol, an emulsifier
otvary izvestkovo-sernye	отвары известково-серные	lime sulfur
paradikhlorbenzol	парадихпорбензол	1,4-dichlorobenzene; p-dichlorobenzene
parizhnaya zelen'	парижная зелень	Paris green
PDB	ПДБ	1,4-dichlorobenzene; p-dichlorobenzene
pentakhlorfenol	пентахлорфенол	pentachlorophenol
pestoks 15	пестокс 15	*N,N'*-diisopropylphosphorodiamidic fluoride
piperonilbutoksid	пиперонилбутоксид	piperonyl butoxide
piperoniltsiklonen	пиперонилциклонен	piperonyl cyclonene
piretriny	пиретрины	pyrethrins
piretrol	пиретрол	an alcohol solution of pyrethrum extract containing 1% pyrethrin I.
piretrum	пиретрум	pyrethrum
pirofos	пирофос	sulfotepp
polikhloridy benzola smeshannye	полихлориды бензола смешанные	mixed benzene polychlorides
polikhlorkamfen	полихлоркамфено	polychlorocamphene [near to toxaphene in composition]
preparat AB	препарат АБ	a preparation based chiefly on copper sulfate
preparat KÉAM	препарат КЭАМ	concentrated anthracene oil emulsion
preparat M-1	препарат М-1	methyl 1-naphthylacetate
preparat M-74	препарат М-74	*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate; Di-Syston
preparat M-81	препарат М-81	*O,O*-dimethyl *S*-2-(ethylthio)ethyl phosphorodithioate
preparat M-82	препарат М-82	*O,O*-dimethyl *S*-2-(methylthio)ethyl phosphorodithioate
preparat 2M–4Kh	препарат 2М–4Х	sodium salt of 2-methyl-4-chlorophenylacetic acid
preparat NIUIF	препарат НИУИФ	*N,N*-diethyl-*m*-toluamide
preparat NIUIF-1	препарат НИУИФ-1	ethylmercuric phosphate and sodium carbonate
preparat NIUIF-2	препарат НИУИФ-2	ethylmercuric chloride and talc
preparat NIUIF-100	препарат НИУИФ-100	parathion
preparat NIUIF-101	препарат НИУИФ-101	parathion
preparat No. 23	препарат № 23	20% dust of ethyl dimethyldithiocarbamate
preparat No. 47	препарат № 47	a mixture of 2-[2-chloroethoxy]ethyl thiocyanate, bis[2-chloroethyl] ether, and bis[2-thiocyanatoethyl] ether
preparat No. 125	препарат № 125	sodium salts of nitrated phenols obtained from tar of semicoked shales or coal
preparat TU	препарат ТУ	sodium salt of 2,4,5-trichlorophenoxyacetic acid
proizvodnye perechnoi (piperovoi) kisloty	производные перечной (пиперовой) кислоты	derivatives of piperic acid [5-(3,4-methylenedioxyphenyl)-2,4-pentadienoic acid]
protars	протарс	14–18% calcium arsenite and diluent
repudin	репудин	preparation similar to dimethyl phthalate

RUSSIAN NAME		ENGLISH NAME OR DEFINITION
Latin Alphabet	Cyrillic Alphabet	
rodan	родан	*p*-aminophenyl thiocyanate
salitsilanilid	салицилaнилид	salicylanilide
sera kolloidnaya	сера коллоидная	colloidal sulfur
sera komovaya	сера комовая	lump sulfur
sera molotaya prirodnaya	сера молотая природная	ground native sulfur
sernistye shlaki	сернистые шлаки	a dark-gray powder containing sulfides of aluminum and other metals; gives off carbon bisulfide on exposure to moisture
sernistyi angidrid	сернистый ангидрид	sulfur dioxide
sernistyi gaz	сернистый газ	sulfur dioxide
sernyi tsvet	серный цвет	flowers of sulfur
serouglerod	сероуглерод	carbon bisulfide
sezamin-maslo	сезамин-масло	sesame oil
smes' bordoskaya sukhaya	смесь бордоская сухая	dry Bordeaux mixture
smes'izvestkovo-sernaya sukhaya	смесь известково-серная сухая	dry lime-sulfur
sol'bar	сольбар	barium sulfide and sulfur
sulema	сулема	mercuric chloride
sul'famat ammoniya	сульфамат аммония	ammonium sulfamate
tabachnye ékstrakty	табачные экстракты	tobacco extract
tetraétilditiopirofosfat	тетраэтилдитиопирофосфат	sulfotepp
tetraétilpirofosfat	тетраэтилпирофосфат	TEPP
tetrametiltiuramdisul'fid	тетраметилтиурамдисульфид	bis(dimethylthiocarbamoyl disulfide)
tiofos	тиофос	parathion
toksafen	токсафен	toxaphene
trikhloratsetat natriya	трихлорацетат натрия	sodium trichloroacetate
trikhlorfenolyat medi	трихлорфенолат меди	copper trichlorophenate
tsianamid kal'tsiya	цианамид кальция	calcium cyanamide
tsianglav	цианглав	a product containing sodium and calcium cyanides with various impurities—carbon, lime, calcium and sodium cyanamides, calcium and sodium sulfides, etc.
tsianistyi natrii	цианистый натрий	sodium cyanide
tsineb	цинеб	zineb
uglerod chetyrekhkhloristyi	углерод четыреххлористый	carbon tetrachloride
vofatoks	вофатокс	a mixture of parathion and methyl parathion
zelen' parizhskaya	зелень парижская	Paris green
zhidkost' bordoskaya	жидкость бордоская	Bordeaux mixture

Diets for Rearing the Sugarcane Borer[1,2]

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ABSTRACT

Mass rearing of the sugarcane borer, *Diatraea saccharalis* (F.), was attempted on a limited scale in the laboratory. Larvae were cultured in cotton-stoppered Erlenmeyer flasks containing sugarcane tops or an artificial agar-base diet. Rearing was done under aseptic conditions.

From a series of experiments in which the amounts of each ingredient in an artificial diet called the "standard diet" were varied, an "improved diet" was formulated. This improved diet contained corn plant powder (an acetone extract from corn plants, 10.0 grams), sucrose (5 grams), cholesterol (0.2 gram), Wesson's salts (1.0 gram), yeast extract (2 grams), Bacto-agar (3.5 grams), and distilled water (125 milliliters).

Results from several experiments indicated that the optimum amount of sugarcane tops for mass rearing should be 15 grams per insect, and that 7½ grams per insect of the improved artificial diet should be adequate for the complete development of sugarcane borer larvae. Ten insects were reared in each 250-milliliter Erlenmeyer flask containing the sugarcane tops. Sugarcane tops appeared to be slightly superior to the improved artificial diet for rearing sugarcane borers.

A study in nutrition of the sugarcane borer, *Diatraea saccharalis* (F.), was undertaken with the following major objectives: (1) To determine whether sugarcane borer larvae could be reared satisfactorily in the laboratory on autoclaved tops of sugarcane plants. (2) To develop an artificial diet for *D. saccharalis* larvae modeled after those used by Beck (1950) and Ishii (1956) for rearing *Pyrausta nubilalis* (Hbn.) and *Chilo suppressalis* (Wlk.), respectively. (3) To develop a mass-rearing technique by which larvae could be reared from eggs to adults without need for periodic care.

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[2] The authors gratefully acknowledge the helpful advice and suggestions contributed by Dr. Norman W. Earle, U. S. Department of Agriculture.

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MATERIALS AND METHODS.—Sugarcane borer eggs were obtained from moths collected as pupae or from full-grown larvae from cane fields. Larvae were fed green corn stems until pupation, and pupae were kept on moist cellucotton in quart-size Mason glass jars. When the moths emerged males were paired with females and 5 or 6 pairs usually were confined together in each oviposition cage. Half-gallon ice cream cartons with bottoms removed and lined with wax paper were used for oviposition cages. These cartons were placed over moist sand and the moths laid their eggs on the wax paper.

Egg masses of *D. saccharalis* were placed on experimental diets less than 24 hours before hatching. Eggs were in the "black-head stage" at this time, and were still on the pieces of wax paper where they had been deposited by the moths. Egg masses were immersed in a 0.1% solution of mercuric chloride for 4 minutes, rinsed in 70% ethyl alcohol, and dropped on the diets in Erlenmeyer flasks. The flasks were then stoppered with plugs of nonabsorbent cotton after the mouths of the flasks and the cotton plugs had been held momentarily in the flame of an alcohol lamp. The entire process of sterilization and introduction of eggs into the rearing flasks was conducted inside a dust-free inoculation chamber.

Larvae were reared on natural and artificial diets. The natural diet was prepared by cutting sugarcane tops into small pieces approximately 2 inches long after removal of the opened whorl and lower leaves. Four or five such pieces were usually obtained from the top of a single sugarcane stalk. Weighed amounts of cane tops were placed in 250- or 500-milliliter Erlenmeyer flasks, which were then plugged with nonabsorbent cotton. A wrapping-paper cap, held in place by a rubber band, was put over the cotton plug to prevent wetting of the plug during autoclaving.

A standard artificial diet was prepared using ingredients and amounts as shown in table 1. The corn plant powder was prepared in the laboratory in a food blender by grinding whole corn plants 24 to 32 inches in height, or the leaves only, with approximately 3 volumes of acetone. The powdered extract was recovered by filtration, resuspended in acetone, filtered again, and dried by spreading to allow evaporation. Many cellulose fibers and coarse particles were removed by sifting through a 16-mesh screen. Bacto-agar was dissolved in distilled water in a boiling water bath for 15 to 20 minutes. Choline chloride was added to the agar-water solution while stirring and was followed by the proper amounts of casein, sucrose, cholesterol, Wesson's salts and yeast extract. After mixing for 3 minutes with an electric stirrer, 0.15 milliliter of a 10% solution of Dowicide-A (sodium O-phenylphenate .4 H_2O) was added and the mixture was stirred again. Finally the corn plant powder was added and mixed thoroughly for about 5 minutes. The diet was then kept at room temperature until coagulation was complete. The desired amounts of diet were weighed and placed in 125- or 250-milliliter Erlenmeyer flasks which were plugged with cotton as described above and prepared for autoclaving.

Table 1.—Artificial diets for rearing the sugarcane borer.

INGREDIENTS	AMOUNTS (GM. OR ML.)	
	Standard Diet	Improved Diet
Corn plant powder	10.00	10.00
Casein	3.50	0.00
Sucrose	5.00	5.00
Cholesterol	0.10	0.20
Wesson's salts	1.00	1.00
Yeast extract	4.40	2.00
Choline chloride	0.05	0.10
Bacto-agar	3.50	3.50
Dowicide-A[a]	0.15	0.15
Distilled water	125.00	125.00

[a] 10% solution in 95% ethyl alcohol.

Sterilization of all diets was accomplished by autoclaving in a 21½-quart capacity pressure cooker. Sugarcane tops were autoclaved at 15 p.s.i. for 20 minutes. Artificial diets were autoclaved at 17 p.s.i. for 20 minutes in some experiments and at 15 p.s.i. for 10 minutes in others. All flasks stood at room temperature for 48 hours after autoclaving to permit the evaporation of excess moisture through the cotton plugs prior to the introduction of eggs into the flasks.

Most of the rearing experiments were conducted in a constant temperature cabinet at 80° F. In some experiments borers were reared in a temperature-controlled room where the average temperature was approximately 80° F., but where occasional fluctuations of as much as 10 degrees above or below this point were experienced.

The effects of the various diets on borer development were evaluated by comparing the results obtained with two or more different diets. The same criteria for evaluation were not used in all experiments. Criteria used included: (1) % pupation; (2) % mortality; (3) average weights of pupae; (4) average weights of larvae; and (5) fecundity of moths as indicated by the average numbers of eggs laid per female and the per cent viability of the eggs. Percentages of mortality and pupation were based on the original numbers of eggs placed on the diets for rearing.

In most experiments the larvae were permitted to feed for 28 to 30 days after which the flasks were opened and the larvae transferred to individual vials containing only moist cotton. They were kept without food in these vials until a succeeding larval moult, pupation, or death. Larvae were considered to have pupated normally on an experimental diet only if they had already done so by the end of the feeding period in the flasks, or if they pupated at the first moult in the glass vials.

Analyses of variance were performed on the data from some experiments to determine whether significant differences had resulted from different treatments. When the variable was a percentage the arcsin transformation was made, as recommended by Snedecor (1956), before the analysis of variance was performed. Duncan's Multiple Range Test, described by LeClerg (1957), was used to determine which means differed significantly from others. In order to prevent contradictions between this range test and the F-test, the range test was used only when the F-test was significant.

RESULTS.—*Natural Diet.*—Sugarcane borer larvae were provided with different amounts of cane tops in rearing experiments to determine the efficacy of autoclaved

sugarcane tops as a complete larval diet, and to determine the amount of cane tops required to rear a specified number of larvae from eggs to adults without periodically opening the rearing containers. Results from two preliminary experiments indicated that rearing flasks should be provided with cane tops at the rate of not less than 10 grams per insect.

On the basis of this information a third experiment was conducted in which 150 grams of C.P. 44-101 sugarcane tops were placed in each rearing flask with 5, 10, 15 and 30 eggs. It was usually necessary to destroy some eggs in each mass with an insect pin in order to have the exact numbers of viable eggs desired. Each flask was opened when pupation was first observed in it which was from 23 to 28 days after the eggs were introduced. Living larvae were then transferred to individual vials without food until a succeeding larval moult, pupation, or death. Results of this experiment are given in table 2 and indicate that mortality was greatest with 5 grams and least with 15 grams of food per insect. The difference in mortality between these two treatments was significant at the 5% level of probability. Seventy-five to 80% of the eggs reached the pupal stage with 15 or 30 grams of food per insect. About half as much pupation was obtained with 5 or 10 grams of food per insect. The amount of pupation obtained with 15 and 30 grams of cane tops per insect differed significantly at the 5% level from that obtained with only 5 grams.

Data given in table 2 also show that the average weights of male and female pupae became smaller as the food supply per insect decreased, and that this effect was most pronounced among females. Females provided with 15 or 30 grams of food per insect were significantly heavier than those with only 5 grams. Males provided with 30 grams per insect were significantly heavier than those with only 5 grams.

Summarized results (table 2) show that female moths from all treatments produced viable eggs. Neither the numbers of eggs laid per female, nor the viability of these eggs, were significantly affected by differences in the amounts of food provided. However, the data do indicate a consistent, although small, decrease in fecundity with each decrease in the amount of food provided.

Table 2.—Results from rearing different numbers of larvae of the sugarcane borer on 150 grams of sugarcane tops, Baton Rouge, Louisiana, June 30, 1959[a].

	Cane Tops per Insect (gm.)			
	5	10	15	30
No. of flasks	4	2	5	4
No. of eggs per flask	30	15	10	5
Mortality (%)[b]	61 A	30 AB	10 B	25 AB
Pupation (%)[b]	30 B	44 AB	80 A	75 A
Weight of ♀ pupae (mg.)[b]	113.4 (17) B	129.9 (8) AB	137.7 (19) A	147.1 (7) A
Weight of ♂ pupae (mg.)[b]	59.9 (9) B	62.9 (3) AB	63.8 (14) AB	66.2 (6) A
No. of eggs per ♀ moth[c]	380.6 (3)	343.0 (2)	404.7 (4)	474.3 (3)
Viability of eggs (%)	95.3	96.5	98.6	99.3

[a] Numbers in parentheses indicate the numbers of individuals upon which each average is based.

[b] All means not accompanied by the same capital letters are significantly different from each other at the 5% level of probability according to Duncan's Multiple Range Test.

[c] An analysis of variance indicated no significant differences at the 5% level of probability.

Table 3.—Results from rearing different numbers of larvae of the sugarcane borer on 50 grams of the standard artificial diet, Baton Rouge, Louisiana, July 7, 1959[a].

	Grams of Diet per Insect			
	1.6	3.3	5.0	10.0
No. of flasks	1	3	3	3
No. of eggs per flask	30	15	10	5
Mortality (%)[c]	43	64	37	33
Pupation (%)[c]	50	31	47	60
Weight of ♀ pupae (mg.)[b]	27.7 (8) A	42.8 (7) A	69.5 (8) B	75.6 (3) B
Weight of ♂ pupae (mg.)[b]	26.9 (7) A	30.2 (6) A	45.0 (6) B	40.3 (6) B

[a,b,] and [c] Same as in table 2.

Three successive generations of borers were reared on autoclaved sugarcane tops with little or no differences in the numbers of eggs laid per moth by moths of the different generations, and with no significant difference in the viability of eggs from first and third generation moths. However, female pupae of the third generation were significantly smaller than those of the first generation.

Artificial Diets.—Table 3 summarizes results from an experiment conducted to determine the amount of the standard artificial diet which should be used to rear a specified number of larvae from eggs to moths in one rearing flask without periodically opening the flask. Different numbers of eggs were placed in flasks which each contained 50 grams of the standard diet. The flasks were opened 30 days after the eggs had been placed in them, and all living larvae were transferred to individual glass vials without food to await another larval moult, pupation, or death.

The data in table 3 show that the least mortality (33%) and the most pupation (60%) occurred in flasks containing 10 grams of diet per insect. In flasks with less food per insect mortality was higher and pupation was lower. However, none of these differences were significant.

Male and female pupae from flasks provided with 5 or 10 grams of food per insect were significantly heavier than those from flasks with only 1.6 or 3.3 grams per insect (table 3). Differences in weights of pupae resulting from different amounts of food provided for the larvae were more pronounced among females than among males.

Seven experiments were conducted to determine whether the standard diet could be improved by changing the proportions of the first seven constituents listed in table 1. Different amounts of each nutrient were used in separate experiments in which all other components of the diet were used in the amounts shown for the standard diet in table 1. Competition for food and space was severe in these experiments. Twenty borer eggs were placed in each 125-milliliter flask with approximately 1.9 grams of experimental diet per insect. The flasks were opened and surviving larvae were counted and weighed after feeding periods of from 20 to 28 days. From these seven experiments an "improved diet" was formulated. The ingredients used and the amounts of each are shown in the last column of table 1. Casein was omitted from the improved diet. The other changes made included increases in the

Table 4.—Results from rearing larvae of the sugarcane borer on different amounts of the improved artificial diet, Baton Rouge, Louisiana, December 19, 1959.[a]

	GRAMS OF DIET PER INSECT		
	5.0	7.5	10.0
No. of flasks	3	2	3
Diet per flask (gm.)	50	75	100
Mortality (%)[b]	60	40	53
Pupation (%)[b]	40	60	47
Weight of ♀ pupae (mg.)[b]	109.3 (6)	104.4 (9)	116.7 (7)
Weight of ♂ pupae (mg.)[b]	58.7 (5)	60.7 (2)	59.5 (7)

[a] and [b] Same as in table 2.

amounts of cholesterol and choline chloride and a decrease in the amount of yeast extract used.

An experiment was then conducted to determine how much of the improved diet should be used to rear a specified number of larvae from eggs to adults in a single rearing flask without periodically opening the flask (table 4). Ten eggs were placed in each of several 250-milliliter flasks which contained different amounts of the improved diet. The flasks were opened 30 days after the eggs were placed in them and all living larvae were transferred to separate vials where they were kept without food until the next larval moult, pupation, or death.

Data presented in table 4 show that there were no significant differences in mortality or pupation among larvae provided with the improved diet when the amounts were varied from 5 to 10 grams per insect. These different amounts of food per insect also had no significant effects on the weights of male or female pupae. Two females, reared from larvae which had fed entirely on the improved diet, laid 390 and 496 eggs, respectively (not shown in table 4), of which 92% and 96% hatched, respectively.

Comparison of Natural and Artificial Diets.—Finally, an experiment was conducted to compare the natural diet of sugarcane tops with the artificial standard and improved diets (table 5). Optimum amounts of each diet, as determined by previous experiments, were used. One hundred fifty grams of sugarcane tops or 50 grams of artificial diet were placed in each 500- or 250-milliliter flask, respectively, with 10 borer eggs per flask. This was equivalent to 15 grams of sugarcane tops or 5 grams of artificial diet per insect. Observations were made after 30 days at which time all living larvae were weighed individually and then transferred to separate glass vials where they were kept without food until another larval moult, pupation, or death. Mortality was highest on the standard diet, intermediate on the improved diet and lowest on the diet of sugarcane tops. Mortality among larvae reared on sugarcane tops was significantly less at the 5% level than that of larvae reared on the standard artificial diet. The average amounts of pupation were greatest on sugarcane tops, intermediate on the improved diet, and least on the standard diet. There was no appreciable difference between the average weights of larvae reared on sugarcane tops and on the improved artificial diet. Larvae (which later developed into females) reared on the standard artificial diet weighed significantly less at the 5% level of probability than those reared on either sugarcane tops or the improved diet.

Table 5.—Comparison of the effects of feeding different natural and artificial diets to larvae of the sugarcane borer, Baton Rouge, Louisiana, September 17, 1959.[a]

	DIETS		
	Sugarcane Tops	Improved	Standard
No. of insects	50	70	50
Mortality (%)[b]	40 A	60 AB	74 B
Pupation (%)[c]	54	34	26
Weight of ♀ larvae (mg.)[b]	143.5 (9) A	141.7 (12) A	96.8 (6) B
Weight of ♂ larvae (mg.)	94.6 (3)	72.0 (6)	61.9 (6)

[a,b,] and [c] Same as in table 2.

DISCUSSION AND CONCLUSIONS.—One of the important objectives of this study was to develop a mass-rearing technique by which *D. saccharalis* could be reared from eggs to adults without need for periodic care or changing of food. The methods of mass rearing used here are believed to have been satisfactory for evaluating the overall efficiency of various combinations of nutrients, numbers of insects, and available space.

Competition for food and/or space was undoubtedly severe in the seven experiments in which rearing flasks were provided with only about 1.9 grams of the standard artificial diet per insect. Results of the experiment summarized in table 3 show that optimum development from eggs to pupae required at least 5 grams of the standard diet per insect. However, the results from this experiment were not available when the seven experiments to determine the optimum amounts of each dietary constituent were conducted. Results from these seven experiments might have been different if larvae had not been overcrowded. However, in spite of these circumstances the results given in table 5 show conclusively that the improved artificial diet was actually superior to the standard diet, since growth was significantly better on it.

All artificial diets used in these studies contained approximately 0.01% by weight of the mold inhibitor, Dowicide-A. No attempt was made to evaluate the effects of Dowicide-A on *D. saccharalis*. However, the concentration used in the artificial diets was approximately one-tenth of that used routinely by Mickey (1958) in culture media for *Drosophila* flies. This small amount of mold inhibitor was probably not very beneficial, and is no longer used in the preparation of the diets described here.

Contamination by fungi was common in these experiments. Rearing flasks were always discarded as soon as fungal growth appeared, and it was normally expected that about 25% of the flasks would be lost during each experiment in this way. This explains the unequal numbers of rearing flasks shown for the different treatments in tables 2, 3 and 4.

The following conclusions were reached:

1. The optimum amount of sugarcane tops for mass rearing was 15 grams of the sugarcane variety C.P.

44-101 per insect. In an experiment in which 500-milliliter flasks were provided with 150 grams of sugarcane tops and 10 borer eggs each, 80% of the eggs developed into pupae.

2. From a series of rearing experiments in which the amounts of each ingredient in an artificial diet called the "standard diet" were varied, an "improved diet" was formulated. It is concluded that 7½ grams of the improved diet per insect is adequate for the complete development of sugarcane borer larvae under mass-rearing conditions, although there is no conclusive evidence to show that 5 grams per insect would not be just as good. In an experiment in which 250-milliliter flasks were provided with 75 grams of the improved diet and 10 borer eggs each, 60% of the eggs developed into pupae.

3. When the relative efficiencies of the improved artificial diet and the natural diet of sugarcane tops were compared, the latter appeared to be slightly superior. Although there were no significant differences between the two, there was less mortality, more pupation, and slightly better growth on the sugarcane tops than on the improved artificial diet.

4. Female sugarcane borer larvae appeared to be more sensitive to deficiencies in their diets than males. Normally full-grown larvae and pupae of females are larger than are those of males. In these experiments the size of the females was reduced more than that of the males by feeding on some diets.

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Differential Sorption of Fumigant Formulations by Wheat and Relative Toxicity to Adult Rice Weevil, *Sitophilus sasakii*[1]

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ABSTRACT

Differential sorption of three fumigant formulations by wheat was determined with the Beckman GC-2 gas chromatograph. Adult rice weevil, *Sitophilus sasakii* (Tak.), mortality was observed. Gas and weevil samples were taken at five different wheat depths in 6-foot towers at 1, 8, 24, 48, 96 and 168 hours following fumigant introduction.

Ratios of CCl_4 to total formulation components were analyzed by an analysis of variance. Changes in CCl_4 properties were studied by orthogonal polynomials.

Variation of CCl_4 proportions in fumigant II (82.39 CCl_4, 17.16 CS_2 and 0.43 n-pentane, per cent by volume) and fumigant III (79.90 CCl_4, 18.06 CS_2, 0.86 petroleum ether and 1.20 SO_2, per cent by volume) was a significant time-quadratic regression changing quadratically with wheat depths. Proportions of CCl_4 in fumigant I (80.11 CCl_4 and 19.89 CS_2, per cent by volume) changed linearly with wheat depths and quadratically with sample times.

Greatest CCl_4 proportions were usually observed in all three formulations near the bottom of wheat columns early in the exposure period. Formulations were approaching equilibrium at 168 hours following introduction. The three fumigants appeared equally toxic to adult weevils.

The differential sorption of various components of a grain fumigant formulation is one of the important variables affecting the toxicity of the fumigant to grain infesting insects. O'Donnell (1958) tested the sorption of carbon tetrachloride (CCl_4) by 12.5% moisture wheat at 80° F. in 4-liter glass bottles and noted sorption equilibrium in approximately 22 hours. El Rafie (1954) reported little increase in carbon disulfide (CS_2) sorption by 13% moisture wheat at 77° F. beyond 24 hours' exposure. Lindgren & Vincent (1959) recirculated 80:20 (CCl_4:CS_2 by volume) through 12.5% moisture whole kernel corn at 70° F. in 53-liter towers for 24 hours and found CCl_4 and CS_2 to have similar sorptive rates.

Our study was conducted to determine differential sorption of CCl_4:CS_2 formulations (with and without certain additives) by 12.0 ± 0.2% moisture wheat at 80 ± 2° F. in towers and relative toxicities of formulations to rice weevil, *Sitophilus sasakii* (Tak.) (Floyd & Newsom 1959), for various sample times.

MATERIALS AND METHODS.—Hard Red Winter wheat harvested in 1952 was cleaned to reduce dockage to less than 1% and stored in 55-gallon steel drums in 200-pound lots. Moisture was determined with a Model G Steinlite moisture tester. Distilled water was added to temper wheat to 12% moisture. Each barrel of wheat was then rotated 30 minutes on each of 5 consecutive days on a mechanical drum roller to provide thorough

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mixing. Wheat was used for experimentation when moisture tested 12.0±0.25% at termination of the tempering process.

Adult rice weevils were 14±1.5 days old. Weevils were reared in Hard Red Winter wheat and stored at 80±2° F. and 70±5% relative humidity.

Fumigations were conducted in nine metal towers, 6 feet long and 8 inches in diameter. Each tower held 6 insect cages at each of 5 levels: 1, 16, 32, 48 and 64 inches below the surface of 105 pounds of wheat. Insect cages were 2½ inches long by 0.75-inch diameter and made of fine mesh stainless steel. Each cage contained 1 gram of wheat and 50 adult weevils. Gas jets, made of 3/16-inch copper tubing, extended to the center of the towers at cage level.

Fumigant-formulations tested were I (80.11:19.89 carbon tetrachloride/carbon disulfide, per cent by volume), II (82.39 carbon tetrachloride, 17.16 carbon disulfide, and 0.43 n-pentane, per cent by volume) and III (79.90 carbon tetrachloride, 18.06 carbon disulfide, 0.86 petroleum ether and 1.20 sulfur dioxide, per cent by volume). Composition of the formulations was determined by gas chromatography.

A 24-hour prefumigation period was allowed prior to introducing fumigants. Fumigated towers received 6.6 ml. (at 20° C.) of the desired formulation which was measured and applied with a 10-ml. tuberculin syringe to the wheat surface. Using 6.6 ml. per 105 lb. of wheat was equivalent to 1 gallon per 1,000 bushels. Preliminary experiments showed a 6.6-ml. dosage produced a wide range of mortalities among weevils for exposure periods tested. Exposure was for 168 hours with gas and insect samples taken at 1, 8, 24, 48, 96 and 168 hours. Gas samples were collected in descending order of depth below wheat surface prior to insect samples. Experiments were replicated three times. Weevils were stored at 80±2° F. in small plastic boxes containing Hard Red Winter wheat. Mortalities were observed 10 days following desired exposures. Weevils that moved were considered alive.

A Beckman GC-2 gas chromatograph was used to obtain quantitative and qualitative gas analyses (Phillips 1956). Prior to use, the gas chromatograph was activated several hours to assure stabilization at 100° C. Helium, used as a carrier gas, was adjusted to flow through the chromatograph column at approximately 83 cc. per minute. The chromatograph column was 6 feet long by 0.25-inch diameter, steel tube containing 15 grams of 42- to 60-mesh crushed firebrick coated with 8 ml. of silicon fluid. Input power was 300 milliamperes and chart speed one-half inch per hour.

Gas samples were removed from towers using 125-ml. glass sample tubes. Neoprene stoppers sealing the tower gas jets were removed, sample tubes attached and gas from the tower allowed to satisfy a 2-mm. vacuum in each sample tube. Gas samples were purged from sample tubes into the gas chromatograph flow system with mercury. Approximately one-half of each gas sample (62.5 ml.) was introduced, thus allowing a second gas analysis of any one sample if necessary.

Various concentrations of carbon disulfide will decompose to form two additional compounds as it passes through the chromatograph column at temperatures of 70°, 100°, 130° and 160° C. (Whitney 1960). Gas samples showing carbon disulfide decomposition as indicated by peaks on the recording paper from questionable sources and by lower carbon disulfide readings with increased concentrations were analyzed by purging the remaining 62.5-ml. gas sample through the chromatograph at 100° C. Diluting the original gas sample with air by a factor of 0.5 reduced carbon disulfide concentration below the critical decomposition level. Dilution factors were checked by comparing carbon tetrachloride readings obtained from both original and diluted gas samples.

A detector and recording system gave characteristic peak locations (elution time) and peak height (gas concentration) for each component. Carbon tetrachloride, carbon disulfide, petroleum ether, normal pentane and sulfur dioxide elution times were approximately 380, 220, 91.5, 100 and 60 seconds, respectively.

Recovery of fumigant components was measured relative to total component recovery and was expressed as a ratio. The sum of recorded ratios for all components from a single sampling must total one; hence the ratios for a two-component formulation are merely complements. Since CCl_4 was the major component in the three formulations studied, CCl_4 proportions were used to summarize the data.

Unequal variances prevent use of analysis of variance on ratios. However, homogeneity of variance tests failed to detect differences in variances within depth in wheat or sample times, probably because of the small range of

Table 1.—Relative proportion of carbon tetrachloride in gas samples for various exposures and wheat depths when introducing 6,600 microliters of fumigant I, II or III into metal towers (59 liter capacity) containing 105 pounds of Hard Red Winter wheat at 12.0±0.1% moisture, <1.0% dockage and 80±2° F.[a]

Time (hrs.)	Per Cent CCl_4 at Various Depths (in.) in Wheat				
	1	16	32	48	64
Fumigant I (80.11% CCl_4, 19.89% CS_2)					
1	84.7	87.9	90.1	93.3	94.4
8	85.7	88.3	90.1	95.8	97.5
24	84.9	85.4	89.1	95.3	95.5
48	83.2	83.6	85.8	89.3	96.3
96	81.7	81.9	83.7	86.6	88.8
168	78.4	85.4	83.2	85.0	88.8
Fumigant II (82.39% CCl_4, 17.16% CS_2, 0.43% normal pentane)					
1	85.0	88.3	90.2	94.0	97.4
8	86.0	88.2	91.6	91.5	91.3
24	81.9	87.8	87.9	91.9	92.5
48	82.0	84.8	86.2	88.6	93.7
96	83.0	84.9	84.2	86.2	87.8
168	83.1	83.3	83.4	84.0	85.2
Fumigant III (79.90% CCl_4, 18.06% CS_2, 0.86% petroleum ether, 1.20% SO_2)					
1	83.6	86.8	89.1	92.7	94.4
8	84.0	86.3	90.2	94.0	96.9
24	83.2	84.0	87.3	91.8	94.6
48	81.7	82.2	84.6	87.3	91.8
96	81.5	79.8	81.1	84.6	87.5
168	81.4	81.7	81.4	81.4	82.8

[a] Average of three replicates.

Table 2.—Analysis of variance of per cent CCl_4 recovered following introduction of three different fumigants into Hard Red Winter wheat.

Source of Variation	Degrees of Freedom	Mean Square ($\times 10^{-5}$)	F	Statistical Significance[a]
Fumigant I (80.11% CCl_4, 19.89% CS_2 by volume)				
Replications	2	53.188		N.S.
Depth in Wheat (D)	4	3,082.758	37.8499	*
Linear	1	12,171.120	149.4361	*
Quadratic	1	22.516	.2764	N.S.
Cubic	1	1.428	.0175	N.S.
Remainder	1	135.970	1.6694	N.S.
Times (T)	5	1,500.609	18.4930	*
Linear	1	6,409.281	78.6927	*
Quadratic	1	578.858	7.1072	*
Remainder	3	171.636	2.1073	N.S.
D×T	20	90.790	1.1147	N.S.
Remainder	58	81.447		
Fumigant II (82.39% CCl_4, 17.16% CS_2, 0.43% normal pentane by volume)				
Replications	2	91.269		
Depth in Wheat (D)	4	1614.124		
Time Intervals (T)	5	1120.372		
D×T	20	107.064	2.0313	*
D Linear×T Linear	1	959.778	18.2100	*
D Linear×T Quadratic	1	13.132	.2492	N.S.
D Quadratic×T Linear	1	75.854	1.4392	N.S.
D Quadratic ×T Quadratic	1	240.862	4.5699	*
Remainder	16	53.228	1.0100	N.S.
Error	56	52.706		
Fumigant III (79.90% CCl_4, 18.06% CS_2, 0.86% petroleum ether and 1.20% SO_2 by volume)				
Replications	2	25.730		
Depth in Wheat (D)	4	2391.160		
Time Intervals (T)	5	1847.217		
D×T	20	115.081	6.6142	*
D Linear×T Linear	1	1848.028	106.2146	*
D Linear×T Quadratic	1	22.965	1.3199	N.S.
D Quadratic×T Linear	1	25.463	1.4635	N.S.
D Quadratic×T Quadratic	1	226.388	13.0116	*
Remainder	16	11.731	.6742	N.S.
Error	58	17.399		

[a] N.S., nonsignificant; *, significant at P = .05.

the CCl_4 ratios, so the data were assumed appropriate for the analysis of variance.

A model I (fixed effects) 5×6 factorial analysis of variance was used on the untransformed data (Snedecor 1956). F-tests were used to evaluate differences in CCl_4 proportions due to depth in wheat and sample times. Each null hypothesis was tested with a .05 probability of a Type I error.

Changes in CCl_4 proportions were studied by use of orthogonal polynomials. Coefficients for computation of the sums of squares for sample times were calculated according to a method by Robson (1959).

RESULTS AND DISCUSSION.—*Sorption.*—With few exceptions relatively larger proportions of CCl_4 than those introduced were observed for all formulations (table 1). The greatest CCl_4 ratios were observed at deeper wheat depths from early sampling times. Fumigant formulation equilibrium was being approached at 168 hours as indicated by small changes in CCl_4 proportions between 96- and 168-hour samplings.

Analysis of gas samples from towers containing fumigant I indicated CCl_4 proportions were changing linearly across depths in wheat and quadratically across time (table 2). Interactions between depths in wheat and time were not significant. The linear change (parallel for all times) in CCl_4 proportions indicates a tendency for higher proportions of CCl_4 at lower wheat depths. Quadratic changes in CCl_4 proportions with increasing time shows the greatest proportion at the 8-hour sampling. Carbon tetrachloride proportions decreased during the remaining 160 hours' exposure (table 2).

Statistical analysis of CCl_4 proportions from fumigant II indicated a significant depth quadratic × time quadratic interaction with higher order interactions nonsignificant (table 2). Apparently the quadratic regression of CCl_4 proportions through grain depths was changing quadratically with sample times. This change of CCl_4 proportions suggests different regressions for each sampling depth in wheat. The quadratic curve with least curvature was observed at the 1-inch depth (table 2), indicating least change in CCl_4 ratios. Gas samples taken at 1-inch wheat depth for 24- and 48-hour samplings had mean CCl_4 recoveries less than the original ratio. A reading of 56.9% CCl_4 was observed from one replication

Table 3.—Per cent mortality of adult rice weevil at various exposures and wheat depths after introducing 6,600 microliters of fumigant I, II, or III into metal towers (59-liter capacity) containing 105 pounds of Hard Red Winter wheat at 12.0 ± 0.1% moisture, <1.0% dockage and 80 ± 2° F.[a]

Time (hrs.)	Per Cent Rice Weevil Mortality at Various Depths (in.) in Wheat				
	1	16	32	47	63
Fumigant I (80.11% CCl_4, 19.89% CS_2)					
1	79.9	57.0	4.7	5.5	2.7
8	100.0	100.0	61.2	10.8	1.4
24	100.0	100.0	100.0	32.2	10.3
48	100.0	100.0	100.0	94.3	19.3
96	100.0	100.0	100.0	100.0	100.0
168	100.0	100.0	100.0	100.0	100.0
Fumigant II (82.39% CCl_4, 17.16% CS_2, 0.43% normal pentane)					
1	61.6	29.7	1.8	1.8	1.7
8	100.0	100.0	67.7	10.2	5.9
24	100.0	100.0	100.0	7.0	4.8
48	100.0	100.0	100.0	96.8	14.1
96	100.0	100.0	100.0	98.6	99.3
168	100.0	100.0	100.0	100.0	100.0
Fumigant III (79.90% CCl_4, 18.06% CS_2, 0.86% petroleum ether, 1.20% SO_2)					
1	89.8	29.9	8.0	7.1	1.3
8	100.0	100.0	74.2	16.8	7.2
24	100.0	100.0	100.0	44.7	7.7
48	100.0	100.0	100.0	99.3	8.4
96	100.0	100.0	100.0	100.0	99.6
168	100.0	100.0	100.0	100.0	100.0

[a] Average of three replicates.

at the 48-hour sampling. This observation appeared erratic and a missing plot technique was used to replace it (changed to 82.0).

Data obtained for fumigant III gave conclusions similar to those for fumigant II (table 2). A significant quadratic change in CCl_4 proportions across depths in wheat was found to change quadratically with sample time. Larger proportions of CCl_4 were generally obtained at 8-hour sampling and at greater wheat depths (table 1).

Mortalities.—Weevil mortalities obtained at various wheat depths and exposures indicated the rate of increasing mortality across time became slower at greater wheat depths (table 3). An exposure of 96 hours was necessary to obtain 100% mortality at all depths in wheat when fumigating with fumigant I while 100% mortality was not attained until 168 hours exposure with fumigants II and III.

It was not realistic to correlate weevil mortality data with gas chromatography results as mortalities reflect amount of some formulation present while gas chromatography results reveal formulation component content. Weevil mortality observed was not dependent on gas analysis ratios data *per se* since formulations varied during exposures.

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A Method and Results of Screening Insecticides against Adult *Hippelates* Eye Gnats[1]

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Abstract

A convenient method for laboratory evaluation of insecticides against adult *Hippelates* eye gnats is described. The insects are exposed for 60 minutes to known deposits of insecticides in Erlenmeyer flasks inverted under a battery of fluorescent lights. By the use of this method, the efficiency of 21 insecticides against *Hippelates collusor* (Townsend) was determined. Three insecticides were also evaluated against *H. robertsoni* Sabrosky. Parathion, dicapthon, and ronnel were the most toxic of all compounds tested. Organophosphorous compounds were in general more toxic than chlorinated hydrocarbon and carbamate insecticides. The susceptibility levels of the two species of eye gnats to organophosphorous compounds were approximately equal.

The recent expansion of cultivated land in the southern California desert areas has considerably increased the natural breeding grounds of *Hippelates* eye gnats. The simultaneous movement of urban populations to the desert resort areas has brought into prominence the need for a thorough investigation of the eye gnat problem and the development of efficient methods of control.

As a result of a project initiated at the University of California Citrus Experiment Station in 1957, a method has been developed for mass rearing of *Hippelates* eye gnats in the laboratory (Mulla & Barnes 1957). This method, as subsequently modified and improved, has made possible the production of unlimited supplies of eye gnats for laboratory use. Valuable information has already been gained on the susceptibility of the immature stages of *Hippelates collusor* (Townsend) to several insecticides applied as soil treatments in the laboratory and in the field (Mulla 1960, Mulla *et al.* 1960).

Treatment of cultivated land with persistent soil insecticides in the Coachella Valley of southiern California, although not yet practiced generally, has pointed out the possibility of substantially reducing the eye gnat population level by this method of control. It is realized, however, that even if soil treatment is practiced on most agricultural land in the area, there will still be a sufficiently high gnat population produced on marginal land, golf courses, and home lawns to require further treatment for adult control in residential and recreational areas. The present study was undertaken to develop a method of screening insecticides on adult eye gnats in the laboratory, and to evaluate the relative toxicity of insecticides that might be used in sprays, aerosols, and baits against these insects.

Materials and Methods.—*Hippelates collusor* was field-collected in Indio, California, in 1957, and *H. robertsoni* Sabrosky in El Toro, California, in 1959. The tests reported here were performed on *H. collusor* adults of the 25th to 35th generations and on *H. robertsoni* adults of the 4th to 6th generations of laboratory rearing. Three- to five-day-old gnats were used since, in preliminary tests with *H. collusor*, the coefficient of variation in response

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to malathion at the LC_{50} was at its lowest level ($S/X = 16\%$) in this age group.

The insects were reared by a method essentially similar to that described by Mulla & Barnes (1957). After emergence the adults were held in metal screen cages (fig. 1) at about 80° F. and were supplied with water and dry proteinaceous food. A separate cage was used each day so that the age of gnats in each cage varied up to a maximum of 24 hours.

The insecticide solutions were prepared in petroleum ether (Skellysolve F) on a weight/volume basis, using technical or purified materials. Insecticides that were not readily soluble in petroleum ether were dissolved first in acetone; further dilutions were made with petroleum ether. One-milliliter volumes of the insecticide solutions to be tested were pipetted into 25-ml. Erlenmeyer flasks and the solvent was evaporated to dryness under a gentle stream of air for 10 minutes. Only the bottom of the flask was wetted by the solution; this area was calculated to measure 12.38 cm^2.

The gnats, in groups of 100 to 150 at a time, were anesthetized with carbon dioxide and 25 insects were aspirated into each treated flask. After the flasks were covered with thin muslin cloth held in place by a rubber band, they were inverted onto a suitable carrying board (fig. 2). They were held for 1 hour at $88 \pm 2°$ F. in a dark box equipped with two overhead fluorescent lamps. Owing to their phototropic behavior, the gnats moved actively over the treated area and were thus exposed to the insecticide. At the end of the 1-hour period the gnats in each flask were anesthetized again with carbon dioxide and transferred into a 75-ml. squat jar having a 50-mesh

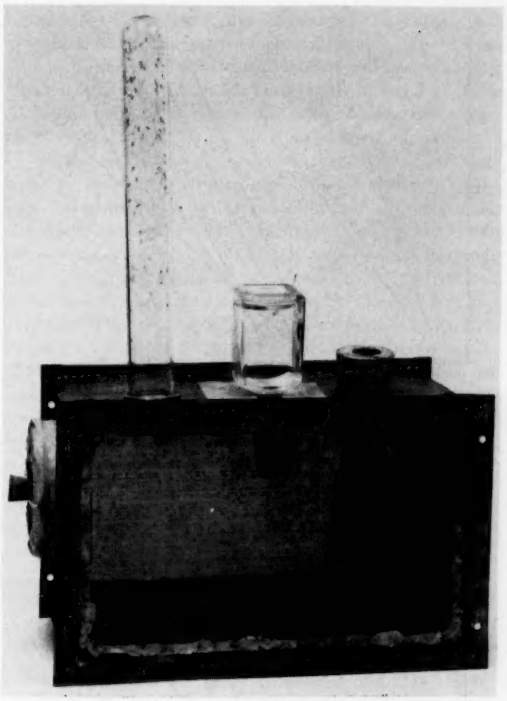


FIG. 1.—Using test tube to remove gnats from holding cage for exposure to insecticides.

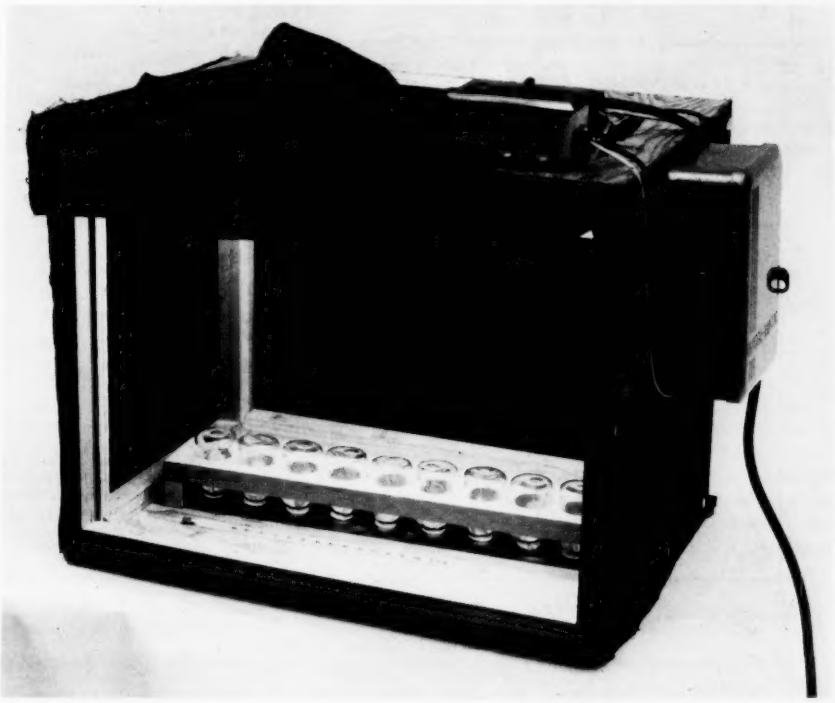
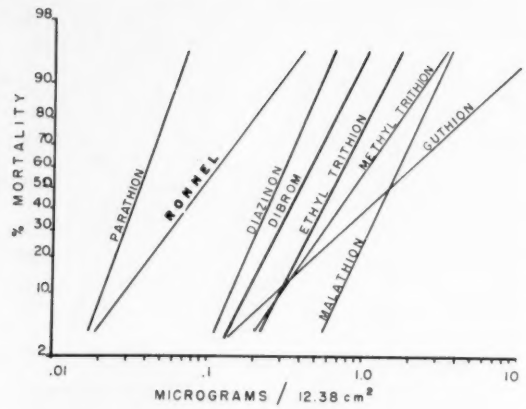
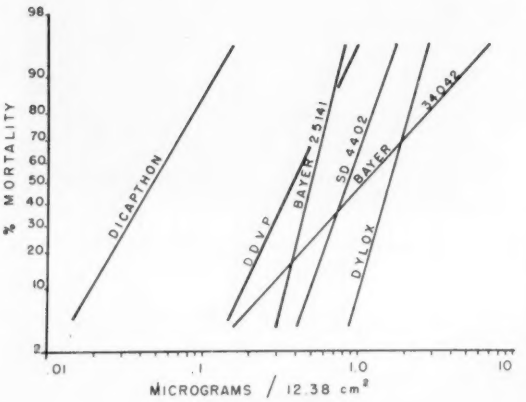


FIG. 2.—Gnats in treated Erlenmeyer flasks held for 1 hour in light box.





FIGS. 3 and 4.—Dosage-mortality regression lines for *Hippelates collusor* exposed for 1 hour to deposits of various insecticides.

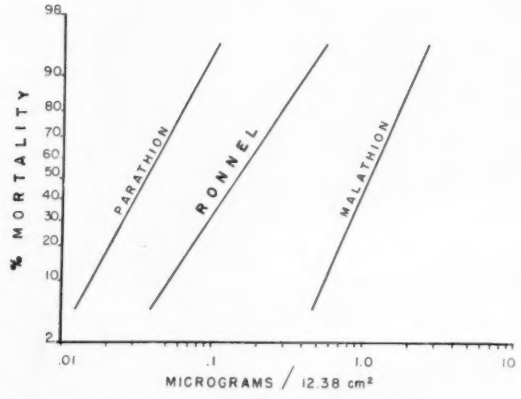


FIG. 5.—Dosage-mortality regression lines for *Hippelates robertsoni* exposed for 1 hour to deposits of various insecticides.

screen top and supplied with a piece of dental cotton roll moistened with 5% sucrose solution. The jars were held for 24 hours in a dark, constant-temperature cabinet maintained at 80° F. and 70% relative humidity. Under these conditions the activity of the gnats was negligible and the mortality in the controls did not exceed 1% on the average.

Mortality counts were taken 24 hours after exposure to the toxicants. The criterion of death was inability to move. At least four concentrations of each insecticide were evaluated and the tests were repeated on four or more different days. Repetition on different dates was necessary since day-to-day variation in susceptibility was greater than variations between replicates of the same date. The average percentage of mortality in probits was plotted against the log of the dosage; the regression lines were fitted by eye. The LC_{50} and LC_{95} for each compound are given in table 1 and figures 3, 4, and 5. The insecticide deposit is expressed in micrograms (1×10^{-6} grams) per 12.38 cm.² which is the entire treated area of a 25-ml. Erlenmeyer flask.

Table 1.—Contact LC_{50} and LC_{95} for *Hippelates collusor* and *Hippelates robertsoni* adults exposed for 60 minutes to deposits of various insecticides in Erlenmeyer flasks.

Insecticide	Micrograms per 12.38 cm.² Treated Surface	
	LC_{50}	LC_{95}
Hippelates collusor		
Parathion	0.036	0.072
Dicapthon	.048	.15
Ronnel	.09	.4
Diazinon[a]	.27	.64
DDVP	.38	1.0
Dibrom[b]	.39	1.06
Bayer 25141[c]	.5	.82
Trithion[d]	.65	1.73
SD-4402[e]	.86	1.73
Methyl Trithion[f]	.87	3.40
Bayer 34042[g]	1.3	6.90
Malathion	1.5	3.65
Guthion[h]	1.5	>10
Dylox[i]	1.6	2.85
Phostex[j]	>10	
DDT	4.8	>10
Dieldrin	>10	
Isolan[k]	>10	
AC-5727[l]	>10	
3-*tert*-butylphenyl *N*-methylcarbamate	>10	
GC 1189[m]	>10	
Hippelates robertsoni		
Parathion	0.036	0.1
Ronnel	.14	.58
Malathion	1.1	2.51

[a] *O,O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate.
[b] 1,2-dibromo-2,2-dichloroethyl dimethyl phosphate.
[c] *O,O* diethyl *O*-*p*-(methylsulfinyl) phenyl phosphorothioate.
[d] S-[*p*-chlorophenylthio)methyl] *O,O*-diethyl phosphorodithioate.
[e] 1,3,4,5,6,7,8,8 octachloro 3*a*,4,7,7*a*-tetrahydro-4,7-methanophthalan.
[f] *O,O*-dimethyl S[(*p*-chlorophenylthio)methyl] phosphorodithioate.
[g] *O*-ethyl *O*-(3-methyl-4-methyl-thiophenyl) *N*-methyl phosphoramidothioate.
[h] *O,O*-dimethyl *S*-4-oxo-1,2,3-benzotriazin-3(4H)-ylmethyl phosphorodithioate.
[i] dimethyl (2,2,2-trichloro-1-hydroxyethyl)phosphonate.
[j] bis(dialkoxyphosphinothioyl) disulfides (alkyl ratio 25% isopropyl, 75% ethyl).
[k] 1-isopropyl-3-methylpyrazolyl-(5)-dimethylcarbamate.
[l] 3-isopropylphenyl *N*-methylcarbamate.
[m] decachloroöctahydro-1,3,4-metheno-2H-cyclobuta[cd] pentalen-2-one.

RESULTS AND DISCUSSION.—Most organophosphorous insecticides tested exhibited a high degree of toxicity to *H. collusor* and *H. robertsoni* adults (table 1). The susceptibility levels of the two species to parathion, ronnel, and malathion were approximately equal. Parathion, ronnel, and dicapthon were considerably more toxic than the other materials tested. Phostex® was the least toxic of the organophosphorous compounds tested, giving practically no mortality at a concentration of 10 micrograms per 12.38 cm².

Mammalian toxicity and residue tolerances are undoubtedly important considerations in adult eye gnat control since treatments will of necessity be applied close to human habitations and on or near agricultural crops. Thus malathion or other less toxic compounds may be more indicated under such circumstances. In this connection, it is noteworthy that from the 1956–57 eradication campaign against the Mediterranean fruit fly (*Ceratitis capitata* (Wied.)) in Florida, although the malathion-treated acreage totaled in excess of 6,727,887 acres (Rohwer 1958), no serious damage has been reported (Morse & Robinson 1957). In preliminary field trials on isolated ranches, malathion used with protein hydrolysate in bait sprays has given temporary but substantial reduction of the gnat population (Mulla 1959).

Chlorinated hydrocarbon insecticides were less toxic than most organic phosphates. DDT, although relatively effective in soil treatments due to its persistence and high degree of toxicity to the immature stages of *H. collusor* (Mulla 1960), was found to possess low toxicity to adult gnats when deposits of 10 micrograms per 12.38 cm.² were evaluated. Addition of a spreader in the test flask (10 micrograms of a chlorinated biphenyl compound) improved spreading and contact with the insects and increased mortality from 66% to 92%. The possibility of resistance to chlorinated hydrocarbon insecticides in *H. collusor* is being investigated.

The carbamate insecticides Isolan,® AC-5727 (3-isopropylphenyl *N*-methylcarbamate), and 3-*tert*-butylphenyl *N*-methylcarbamate, demonstrated very low toxicity to *H. collusor*, the relevant LD_{50} values being greater than 10 micrograms per 12.38 cm². These insecticides manifested, however, considerable knockdown effect, the KD_{50} values for 10 micrograms of Isolan and AC-5727 being 3.5 and 6 minutes, respectively, compared with 32 minutes for an equal deposit of malathion. Under the conditions of these tests, knockdown prevented further exposure to the insecticide and thus resulted in lower mortality than could be obtained with a continuous 60-minute exposure. The mortality obtained with 10-microgram deposits of Isolan and 3-isopropylphenyl *N*-methylcarbamate was 24% and 16%, respectively, when the exposure was interrupted by knockdown, compared with 42% and 32%, respectively, when exposure was continuous for 60 minutes. Although the percentage of mortality was thus doubled, it is still too low to hold any promise for use of these carbamates in gnat control. Knockdown was also very pronounced with some of the organic phosphates, especially Dibrom and DDVP.

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The Recovery of Genes Controlling Corn Borer Resistance in a Backcrossing Program[1]

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ABSTRACT

A modified backcrossing procedure incorporated desirable morphological characters into a dent corn inbred line Oh45, resistant to leaf feeding of first-brood European corn borer (*Pyrausta nubilalis* (Hbn.)). Outcrossing, to a corn borer susceptible single cross, and then selfing with selection fixed the desired characters. With another backcrossing to the inbred line and continued selfing, three lines designated as Oh45A, Oh45B, and Oh45C were developed as resistant to first-brood leaf feeding as the recurrent parent. One of these lines may be released in the future as a partial replacement for Oh45.

Resistance to the European corn borer (*Pyrausta nubilalis* (Hbn.)) has had an important role, since 1926, in the cooperative Ohio-U. S. Department of Agriculture corn-breeding program. From this research has come, among others, such resistant inbred lines as Oh07, Oh40B, Oh41, Oh43, Oh45, and Oh51A. These lines have entered importantly into useful hybrids, not only in Ohio but also across the Corn Belt, and in foreign countries. Of the 15 recommended Ohio hybrids, four have the single cross of sister lines Oh43 and Oh45 as the pollinator

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Tests made in 1959 were in cooperation with E. J. Dollinger, corn geneticist, Ohio Agricultural Experiment Station, Wooster.

FIG. 1.—WF9 check plot showing an extremely high level of plant infestation; first 10 plants infested with 10 egg masses per plant: (a) plants in foreground greatly stunted by feeding of first-brood corn borer larvae; background plants not infested, and (b) close-up of plot. 1959.

parent. Severe injury by corn borers is prevented by the use of these hybrids. Inbred Oh43 also contributes to high yields of hybrids under widely different environmental conditions. Stringfield (1959) demonstrated that inbred Oh45 contributes overtight husks, short shanks, inadequate pollen production, and sensitivity to heat and moisture. The present paper reports a modified backcrossing procedure to improve Oh45.

Inbred lines carrying corn borer resistance have been isolated by two methods: (1) selfing in open pollinated varieties or crossing of inbred lines, and (2) backcrossing. The program has been directed primarily toward breed-

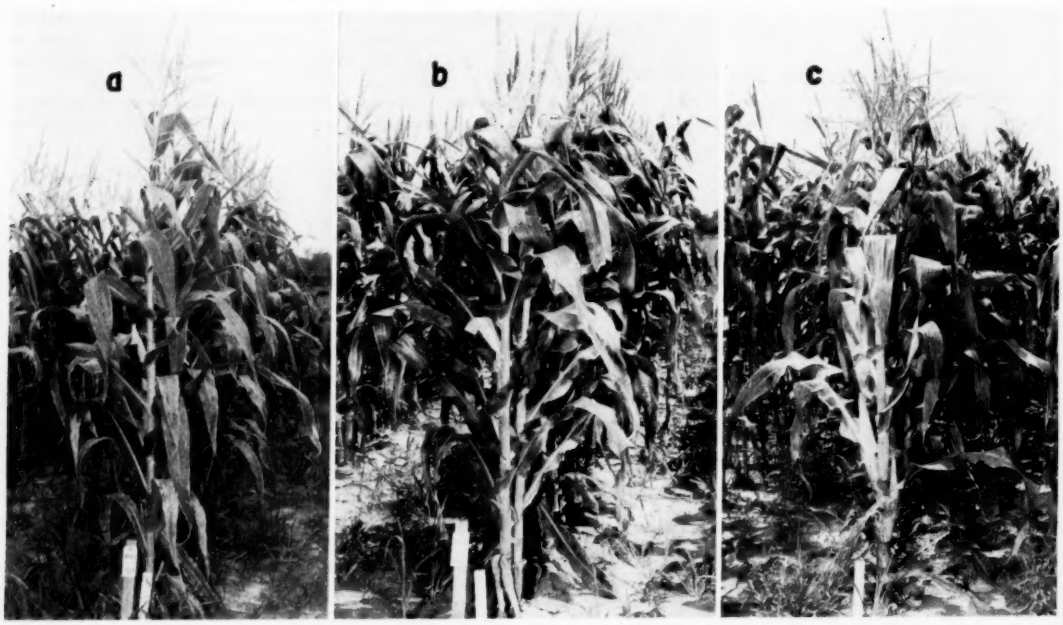

FIG. 2.—Corn borer resistant plots showing little leaf injury by larvae feeding in the whorl; first 10 plants infested with 10 egg masses per plant: (a) Oh45B, (b) Oh45C, and (c) Oh45, recurrent parent. 1959.

Table 1.—Frequency distribution of first-brood corn borer segregates among cultures of two families [(M14×CI.187-2)×Oh45]S_4×(M14×CI.187-2) and [(M14×CI.187-2)×Oh45] S_4×Oh45.

Recurrent Parent	Generation After Backcross	Year	Number of Cultures Tested	Per Cent of Segregates Classed as: Resistant	Intermediate	Susceptible
(M14×CI.187-2) Oh45	S_0	1953	36	—	50	50
	S_0	1953	32	53	47	—
	S_1	1954	16	44	56	—
	S_2	1955	15	53	47	—
	S_3	1958	15[a]	100	—	—
	S_0	1959	5[b]	100	—	—

[a] Four cultures were Oh45A.
[b] Two cultures were Oh45B and three cultures Oh45C.

ing for resistance to the first brood and tolerance to the second brood (Dicke 1954).

METHODS.—In 1947, Oh45 (designated as recurrent parent) was outcrossed with M14×CI.187-2 (designated as donor parent) which has an abundant, loose husk, well-developed shanks, adequate pollen production, but a high susceptibility to the first-brood infestation. A single cross, rather than an inbred line, was chosen as donor parent in the belief that the greater genetic diversity of the three-parent population would provide improved selection over a two-parent one.

In 1948 the outcross, (M14×CI.187-2)×Oh45, was both selfed and backcrossed to Oh45. The backcross progeny appeared to offer too few donor morphological characteristics for effective selection. Selfing with selection was continued to establish the donor shank and husk characters along with plant and grain characters of Oh45. After the S_4 (fourth selfed) generation, crosses were made using both Oh45 and M14×CI.187-2 as test-cross parents to establish the genetic drift in each S_4 culture. Those that had drifted toward Oh45 should have exhibited relatively low heterosis when crossed with Oh45 and relatively high heterosis when crossed with M14×CI. 187-2, and vice versa. Test-cross stands were poor and the expected negative correlation failed to develop.

The test crosses were artificially infested, simulating a first-brood infestation, and several of the cultures were selected for continuance based on appearance, test-cross performance of agronomic characters, and reaction to the borer. Both types of test crosses from the selected cultures were then selfed; for example [(M14×CI.187-2)×Oh45] S_4×Oh45, and [(M14×CI.187-2)×Oh45] S_4×(M14 ×CI.187-2). In the succeeding seasons S_0, S_1, S_2, S_3 and S_0 generation cultures from the backcross to Oh45 were artificially infested and appropriate discards made. Cultures were grown in single rows of 38 planted seeds.

Plants were infested artificially by dropping four egg masses, or approximately 80 eggs, in the whorl during the mid-whorl stage of plant growth in two applications of two masses each spaced 3 to 7 days apart; 10 plants in each plot were infested. A rating system, reflecting the pattern of leaf injury, as described by Guthrie *et al.* (1960), was used to evaluate the relative degree of resistance to leaf feeding. Leaf injury was rated as follows: a light infestation showing small round pin-hole, or slightly elongated lesions, from 1 to 3; a moderate number of medium-sized elongated lesions, 4 to 6; and numerous large elongated and round lesions, 7 to 9. Susceptible and resistant leaf-feeding patterns are shown in figures 1 and 2. Plants were first selected on a plot basis before pollination to eliminate the susceptible cultures. Individual plant selections were then made within the plots graded resistant.

RESULTS.—Frequency distributions in three categories of resistance are presented in table 1. When M14×CI. 187-2 was used as the recurrent parent, the distribution of S_0 backcross cultures carrying genes from the susceptible and resistant parents was in a ratio of 1 to 1 of intermediate and susceptible segregates. With Oh45 as the recurrent parent, the distribution of S_0 cultures was approximately in a ratio of 1 to 1 of resistant and intermediate segregates; this was doubtless somewhat biased by selection for the resistance genes of the recurrent parent.

Table 2.—First-brood European corn borer leaf-feeding ratings of Oh45 recoveries [(M14×CI.187-2)×Oh45] S_4×Oh45, obtained at Toledo, Ohio, in 1953–54 and Wooster, Ohio, in 1955–59.

Year	Segregate	Leaf-Feeding Rating
1953	S30-1-1-2-Oh45	2
	S60-1-1-1-Oh45	3
	Checks:	
	Oh43	2
	WF9	7
1954	S60-1-1-1-Oh45-S_2	3
	Checks:	
	Oh43	3
	WF9	9
1955	S30-1-1-2-Oh45-S_1	4
	S_2	2
	S_3	3
	S_4	3
	S60-1-1-1-Oh45-S_2	4
	Checks:	
	Oh43	3
	WF9	9
1958	S30-1-1-2-Oh45-S_2-2-1-1-1	2
	2-1	2
	2	2
	3-1[a]	2
	2[a]	3
	3[a]	3
	4[a]	3
	S30-1-1-2-Oh45-S_2-2-1-1	2
	Oh45-S_2-1-2-1-1[b]	2
	2	2
	2-1	2
	2-2-2	2
	S60-1-1-1-Oh45-S_2-1-1-1-1[c]	2
	2	2
	3	3
	Checks:	
	Oh43	2
	WF9	9
1959	S30-1-1-2-Oh45-S_2-1-2-1-1-2[b]	1
	3[b]	1
	S60-1-1-1-Oh45-S_2-1-1-1-1-1[c]	1
	2[c]	1
	3[c]	1
	Oh45 (recurrent parent)	2
	WF9 (check)	9

[a] Given permanent designation Oh45A.
[b] Given permanent designation Oh45B.
[c] Given permanent designation Oh45C.

Generations S_1–S_6 showed the effect of continued selection for resistance. The marked and rapid effect of selection pressure in this material suggested that a major fraction of the corn borer resistance was rather simply inherited.

The ratings of specific segregates, resistant checks Oh43 and Oh45, and susceptible check WF9 are presented in table 2. In this table S30 and S60 are two of the original S_1 plants which were sources of the most desirable segregates. The numbering system used in table 2 designates the ear number selected in each selfed generation; for example, in 1955, S30-1-1-2-Oh45-S_1 indicated that S30 was the ear selected in the first generation, ear number 1 was selected in the second generation, ear number 1 in the third generation, and ear number 2 in the fourth generation. After the fourth selfed generation this segregate was backcrossed to Oh45, and S_1 refers to the ear selected from the first selfing after backcrossing. Three of the best lines from table 2, considering borer resistance and other characters, were given permanent designations of Oh45A, Oh45B, and Oh45C in 1958.

In 1959 the Oh45B and Oh45C lines were exposed to much stress. The first 10 plants in each plot were artificially infested with 10 egg masses, or approximately 200 eggs per plant, in five applications of two masses from the early- to late-whorl stage of plant development but before tassel emergence. Inbreds Oh45 and WF9 were included as checks. A high level of larval establishment and survival resulted in the susceptible WF9 check plot. The infested plants of WF9 were injured to the extent that they did not produce an ear (figures 1a and b). Inbreds M14 and CI.187-2, which were components of the donor parent, were not included in the 1959 test since many comparisons have shown that they are nearly as susceptible as WF9. Oh45B and Oh45C (figures 2a and b and table 1) were as resistant as the recurrent parent, Oh45 (figure 2c and table 1). Compared with Oh45, these recoveries have improved shank, husk, and pollen-production qualities, but the new lines are not otherwise precise duplicates of Oh45.

Oh45A, Oh45B, or Oh45C may be released in the future as a partial replacement for Oh45, or they may be recombined and selected further.

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Efficient Utilization of Paulins in Fumigation and as Covers for the Protection of Storage[1]

MYLES F. BOWEN[2]

ABSTRACT

Formulas are given for determining the dimensions of the ideal stack which will give maximum volume under paulin covers used for fumigation or for the protection of storage. Some applications of the formulas are discussed, and a table is included which gives the ideal stack dimensions for paulins of various sizes and shapes when different amounts of overlap are provided for sealing. The study demonstrates the following: (1) The square is the best shape of paulin if the highest ratio of stack volume to paulin area is to be achieved. (2) The base of the ideal stack will resemble the shape of the paulin used to cover it. (3) Large paulins are innately more efficient than smaller ones because of the surface-volume relationship. (4) When two or more paulins are combined, greatest efficiency results if they are combined along the dimension that makes the difference between the length and width of the combined cover as small as possible, *i.e.*, when the combined cover is as nearly square as possible.

The fumigation of stored products and other materials under paulins frequently involves estimates of the arrangement of the material in the stacks for best utilization of the paulins available for the job. This is especially true when large lots of commodity requiring several separate stacks are involved, or when the size of the paulin is barely large enough to cover the volume of material to be treated. Failure to stack the material so as to make fullest utilization of the paulin will often make two fumigations necessary, whereas with the right arrangement one would suffice.

Whenever materials are stacked for fumigation or for storage under paulins, careful attention should be given to the sizes and shapes of the paulins available before determining the dimensions of the stacks to be made in order to utilize the covers most efficiently. The primary intention of this study is to provide a ready means for determining the optimum paulin-stack relationship which will be useful in connection with the growing popularity of the paulin method of fumigation.

DEVELOPMENT OF FORMULAS.—For any given paulin, there is an ideal stack which it will fit, and at the same time cover a maximum quantity of the product being fumigated. If the dimensions of the stack deviate very much from the ideal, either there may not be enough overlap to permit adequate sealing about the base of the stack, or much of the paulin may be wasted in useless overlap. In any event, departures from the ideal dimensions will reduce the volume in the stack regardless of the fitness of the overlap.

Sufficient overlap to permit a gastight seal is essential

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Table 1.—Dimensions of stack for maximum volume under fumigation paulins of various shapes and sizes.

Paulin Dimensions		Stack Dimensions for Maximum Volume (ft.)			Maximum Volume
Actual Width ×Length (ft.)	Effective[a] Width ×Length (ft.)	Height	Width	Length	(cu. ft.)
25×25	23×23	3.83	15.33	15.33	900
	21×21	3.50	14.00	14.00	686
	19×19	3.17	12.67	12.67	509
25×30	23×28	4.19	14.62	19.62	1,202
	21×26	3.85	13.30	18.30	937
	19×24	3.51	11.98	16.98	714
30×30	28×28	4.67	18.67	18.67	1,627
	26×26	4.33	17.33	17.33	1,300
	24×24	4.00	16.00	16.00	1,024
30×35	28×33	5.03	17.94	22.94	2,070
	26×31	4.69	16.62	21.62	1,685
	24×29	4.36	15.28	20.28	1,351
30×40	28×38	5.31	17.38	27.38	2,527
	26×36	4.97	16.06	26.06	2,080
	24×34	4.62	14.76	24.76	1,688
35×35	33×33	5.50	22.00	22.00	2,662
	31×31	5.17	20.67	20.67	2,209
	29×29	4.83	19.34	19.34	1,807
35×40	33×38	5.87	21.26	26.26	3,277
	31×36	5.54	19.92	24.92	2,750
	29×34	5.20	18.60	23.60	2,283
40×40	38×38	6.33	25.33	25.33	4,061
	36×36	6.00	24.00	24.00	3,456
	34×34	5.67	22.67	22.67	2,914
40×45	38×43	6.71	24.58	29.58	4,879
	36×41	6.38	23.24	28.24	4,187
	34×39	6.04	21.92	26.92	3,564
40×50	38×48	7.02	23.96	33.96	5,712
	36×46	6.68	22.64	32.64	4,936
	34×44	6.34	21.32	31.32	4,234
45×45	43×43	7.17	28.67	28.67	5,893
	41×41	6.83	27.33	27.33	5,102
	39×39	6.50	26.00	26.00	4,394
45×50	43×48	7.55	27.90	32.90	6,930
	41×46	7.21	26.58	31.58	6,052
	39×44	6.88	25.24	30.24	5,251
50×50	48×48	8.00	32.00	32.00	8,192
	46×46	7.67	30.67	30.67	7,215
	44×44	7.33	29.34	29.34	6,310

[a] Dimensions after allowing for overlap for sealing. Upper figures correspond to 1-foot overlap; middle figures correspond to 2-foot overlap; lower figures correspond to 3-foot overlap.

to effective fumigation. Adequate sealing may be had with as little as 1 foot of overlap, but in practice, $1\frac{1}{2}$ to 2 feet or more is preferable depending somewhat on the size of the stack. Obviously it should be as small as practicable. The effect of overlap is illustrated in table 1 by comparing the maximum volumes that can be covered by the same paulin using 1-foot, 2-foot or 3-foot overlap.

The dimensions of the ideal stack for any paulin may be computed directly from the formulas that follow. The formula derived for computing the height h of stack for maximum volume under the paulin is,

$$h = \frac{L + W - \sqrt{L(L - W) + W^2}}{6} \quad (1)$$

in which L and W are the effective length and width of the paulin, *i.e.*, the length and width after deducting for overlap at the bottom for sealing.[3] The length l and the width w of the ideal stack are then determined by the following relationships:

$$l = L - 2h \quad (2)$$

$$w = W - 2h \quad (3)$$

If the length and width of the paulin are equal, it becomes a square with effective side $A = L = W$. Equation (1) for h then reduces to,

$$h = \frac{A}{6} \quad (4)$$

and the base of the stack of maximum volume that can be covered by the paulin will also be a square with side a such that,

$$a = A - 2h = \frac{2A}{3} \quad (5)$$

APPLICATION TO FUMIGATION.—Table 1 shows some of the many possible sizes and shapes of paulins, together with the dimensions of the ideal stack for each. Values in the table were computed by substitution in the above equations. In practice, these dimensions can seldom be duplicated exactly. They will, however, be useful as a guide to stacking commodity for paulin fumigation; the objective being to approximate as nearly as practicable the ideal dimensions. Some adjustment to these dimensions will be needed to compensate for a diffusion dome at the top of the stack to permit even distribution of the fumigant.

Fumigation paulins may be purchased in any desired dimensions, but the square is the best shape if the highest ratio of stack volume to paulin area is to be achieved. This fact, which can be demonstrated mathematically, is suggested by the figures in table 1. For example, the 30′×40′ and the 35′×35′ paulins have about the same area, the latter being about 2% larger than the former. However, the 30′×40′ paulin after allowing for a 2-foot overlap will cover only 2,080 cubic feet of stack at maximum volume; whereas the 35′×35′ paulin will cover 2,209 cubic feet, or 6% more.

In this connection, observe that the shape of the base of the ideal stack resembles the shape of the paulin used to cover it after making proper allowance for stack height and overlap for sealing. That is, if the paulin is square, the base of the ideal stack will also be square; if the paulin is oblong, the base of the ideal stack will be oblong. Specifically, the difference between the length and the width of the ideal stack will be the same as the difference between the length and the width of the paulin. Stated algebraically, $l - w = L - W$.

Further example of increased efficiency, resulting from a change of size and shape, is provided by considering the

[3] Note that the effective dimension is the actual dimension minus two times the overlap.

30′×40′ paulin. Adding 10 feet to the width, making it 40′×40′, will increase the area by 33% but at the same time it will increase by 73% the maximum volume that it will cover for fumigation when a 3-foot overlap is provided for sealing. With only a 2-foot overlap or a 1-foot overlap, the maximum volume will be increased by 66% and 61%, respectively.

Not all of the increased efficiency of the 40′×40′ over the 30′×40′ paulin can be attributed to the change in shape from oblong to square. Most of it stems from the fact that large paulins are innately more efficient than smaller ones, simply because of the well-known relationship of surface to volume, namely, that surface varies directly as the square of a dimension, whereas volume varies directly as the cube.

Frequently the problem is to determine whether a paulin will cover an existing stack of commodity without going to the trouble and expense of moving and restacking it. For example, assume a stack of material 15 feet wide, 20 feet long, and 10 feet high needs fumigation. A 40′×40′ paulin is available for the job. Will it cover the stack after allowing 2-foot overlap on all sides for sealing? This can be determined by solving equations (2) and (3) for L and W, respectively. Thus,

$$L = l + 2h \quad (6)$$

$$W = w + 2h \quad (7)$$

from which by substitution,

$$L = 20 + 2 \times 10 = 40 \text{ feet}$$

$$W = 15 + 2 \times 10 = 35 \text{ feet}$$

Since the effective length and width of the 40′×40′ paulin is only 36 feet, after allowing for 2-foot overlap, it is obvious that the stack cannot be adequately covered. The commodity will have to be restacked or have the height and/or length decreased so that substitution in equation (6) gives 36 feet or less. The entire lot may be covered by restacking it to the dimensions for maximum volume indicated in table 1 for a 40′×40′ paulin. This is obvious, since the stack (15′×20′×10′) contains only 3,000 cubic feet, while table 1 shows that as much as 3,456 cubic feet may be covered with a 40′×40′ paulin, allowing 2-foot overlap, if the material can be stacked to the ideal dimensions (24′×24′×6′). In practice it may not be feasible to build the stack to the desired dimensions because of limitations imposed by the size of the unit package or the dimensions of the pallets on which the commodity is loaded for handling with forklift or other materials handling equipment. If additional paulins are available, the simplest procedure will be to join two paulins together so as to cover the stack in place.

APPLICATION TO PROTECTION OF STORAGE.—The same principles will apply to the use of paulins as covers to protect storage from dust or the weather. For these uses, however, the cover need not be gastight as in fumigation. Frequently no overlap at the bottom is necessary, or only the top and part of the sides of the stack require cover to protect it from inclement weather. When no overlap at the bottom is needed, the top and sides of the stack will be completely covered and the effective and actual dimensions of the paulin will be the same. In this instance, and when some overlap at the bottom is needed to keep out dust or to secure the paulin, equations (1) through (7) are applicable. If only the top and part of the sides need to be covered, equations (8) through (10), which are given later, are appropriate.

Table 2.—Dimensions of stack for maximum volume under paulin covers.[a]

Paulin Dimensions		Stack Dimensions for Maximum Volume (ft.)			Maximum Volume
Basic Size Width ×Length (ft.)	Multiple of Basic Size[b] Width ×Length (ft.)	Height	Width	Length	(cu. ft.)
12×17		2.31	7.38	12.38	211
	17×23 (2L)[c]	3.22	10.56	16.56	563
	12×33 (2W)	2.68	6.64	27.64	492
	17×34 (3L)	3.59	9.82	26.82	946
20×20		3.33	13.33	13.33	592
	20×39 (2)	4.20	11.60	30.60	1,491
	20×58 (3)	4.50	11.00	49.00	2,426
20×40		4.23	11.54	31.54	1,540
	39×40 (2L)	6.57	25.86	26.86	4,564
	40×58 (3L)	7.76	24.48	42.48	8,070
	20×79 (2W)	4.64	10.72	69.72	3,468
17×40		3.70	9.60	32.60	1,158
	33×40 (2L)	6.00	21.00	28.00	3,528
	40×49 (3L)	7.30	25.40	34.40	6,378
	17×79 (2W)	4.00	9.00	71.00	2,556
15×60		3.49	8.02	53.02	1,484
	29×60 (2L)	6.17	16.66	47.66	4,899
	43×60 (3L)	8.24	26.52	43.52	9,510
	57×60 (4L)	9.74	37.52	40.52	14,808

[a] Top and all sides completely covered; no overlap at bottom.
[b] Dimensions of cover resulting from combined paulins after allowing 1-foot overlap along each seam.
[c] Numbers in ( ) denote number of paulins and dimension along which joined.

The dimensions of the ideal stack for some of the standard paulins used as protective covers by the U. S. Army Quartermaster Corps are given in table 2. The values in this table are based on the assumption of full coverage of the top and sides with no overlap at the bottom. Since two or more paulins are frequently used in covering stacked material, data are also given for a few multiples of the basic sizes after allowing for 1-foot overlap along the seams.

From table 2 it will be noted that when two or more paulins are used, the greatest efficiency results when they are combined along the dimension that makes the difference between the length and width of the combined cover as small as possible; *i.e.*, when the shape of the combined cover is as nearly square as possible. This is illustrated by comparing the maximum volume (4,564 cubic feet) that can be covered by two 20′×40′ paulins combined along the length, with the maximum volume (3,468 cubic feet) that can be covered by two of the same paulins combined along the width. The former volume is 32% greater than the latter.

If only the top and part of the sides of the stack require cover, the overlap may be considered as extending from the top down the sides. The height, length and width may then be varied as needed to give the desired overlap at the top. For this usage, the following relationships will apply:

$$l = L - 2X \quad (8)$$

$$w = W - 2X \quad (9)$$

in which L and W are the actual length and width of the paulin; l and w are the dimensions of the base of the stack, and X is the overlap at the top. Now, maximum volume protected by the paulin will be achieved when it covers the top of the stack with the required overlap and the stack is infinitely high; or, for practical purposes, when the stack is as high as feasible considering safety, the cost of stacking to various heights, available space, or other limitations.

If it is desired that the overlap X cover P per cent of the sides of the stack, the height will be limited to

$$h = \frac{100X}{P} \qquad (10)$$

To illustrate the application of equations (8) through (10) assume: (1) that storage is to be arranged in stacks which can be covered by two $20' \times 40'$ paulins combined along the length; (2) that a 5-foot overlap is needed at the top; and (3) that the overlap must cover at least 50% of the sides of the stack. What should be the dimensions of the stack?

The dimensions of the combined paulin, assuming 1-foot overlap along the seam, will be $39' \times 40'$. The length of the stack by substitution in equation (8) is then,

$$l = 40 - 2 \times 5 = 30 \text{ feet}$$

The width from equation (9) is,

$$w = 39 - 2 \times 5 = 29 \text{ feet}$$

The height from equation (10) then must not exceed,

$$h = \frac{100 \times 5}{50} = 10 \text{ feet}$$

and the stack will contain $29 \times 30 \times 10 = 8{,}700$ cubic feet.

When better drainage is required than direct application of the paulins will allow, some adjustment to the ideal dimensions given in table 2, or computed from the various equations, will be necessary to compensate for the additional height at the center needed to provide slope for drainage.

Oviposition Response of the Army Cutworm, *Chorizagrotis auxiliaris*, to Different Media[1]

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ABSTRACT

Moths of the army cutworm, *Chorizagrotis auxiliaris* (Grote), (Lepidoptera: Noctuidae) responded ovipositionally to differences in soil color, texture, and depth. Number of eggs laid increased with amount of light reflected. A combination of loose surface over a solid substrate was required. A light-colored sand fulfilled all requirements and was preferred over soil. Soil was acceptable when a loose surface of correct depth was provided. Solid surfaces were refused.

The army cutworm, *Chorizagrotis auxiliaris* (Grote), (Lepidoptera: Noctuidae) is an important pest of wheat, barley, alfalfa, and other crops in the Great Plains. Jacobson & Blakeley (1959) discuss factors favoring general increases in army cutworm populations. Within an outbreak area there also are often large differences in populations between adjacent fields. Pruess (1961) found that winter barley usually had higher populations than adjacent wheat fields. These differences must be caused either by differences in larval establishment or by number of eggs laid. The latter is considered in this study.

Little information is available on the oviposition habits of this cutworm moth. Johnson (1905) was undoubtedly in error in reporting eggs laid on foliage. Cooley (1916) reported that eggs may be laid on the underside of clods, buried below the surface in loose soil, or laid on the surface in dry, hard fields. Strickland (1916) concluded that eggs were laid in, rather than on, soil. Blakeley *et al.* (1958) found a loose soil suitable for oviposition.

To determine the conditions suitable and favored for oviposition, a variety of soils and other media were compared for attractiveness as oviposition sites.

METHODS.—Moths of the army cutworm were collected in a black light trap during September and October, 1958, and refrigerated at 35° F. until needed. Most moths were used within a week after collection, but a few were held as long as a month without serious mortality. Those stored for prolonged periods laid fewer eggs.

Tests were conducted in a laboratory having windows along the entire north wall. Cubical cages, either 2 or 3 feet on a side, were used to contain the test media and moths. Except for the 1-inch framework and a plywood bottom, these cages were of screencloth.

Soils or other test media were placed in 8-cm. lids of circular cardboard cartons. These dishes were filled with test media to a depth of approximately one-half inch except in tests comparing specified depths. When shallower depths were used, a layer of plaster of Paris or hard soil was used in the bottom of the dish to equalize surface levels.

The media used were a coarse sand consisting of particles passing an 18-mesh screen but retained by a 40-mesh screen; coarse perlite[2] of the above size; and fine perlite, sand, and soil all finer than 60-mesh. The fine sand was actually somewhat coarser than the soil and much lighter in color as shown in figure 1 which depicts several media used. The soil was Holdrege very fine sandy loam taken from the top 4 inches of a cultivated field.

Wheat straws were used to simulate a mulch in several tests. Straws were cleaned of leaf sheaths and cut in pieces averaging $1\frac{1}{4}$ inches in length. Colored straws used in several tests were dyed by boiling in Rit.[3] Sand was colored with a coal tar dye food coloring. The dye was

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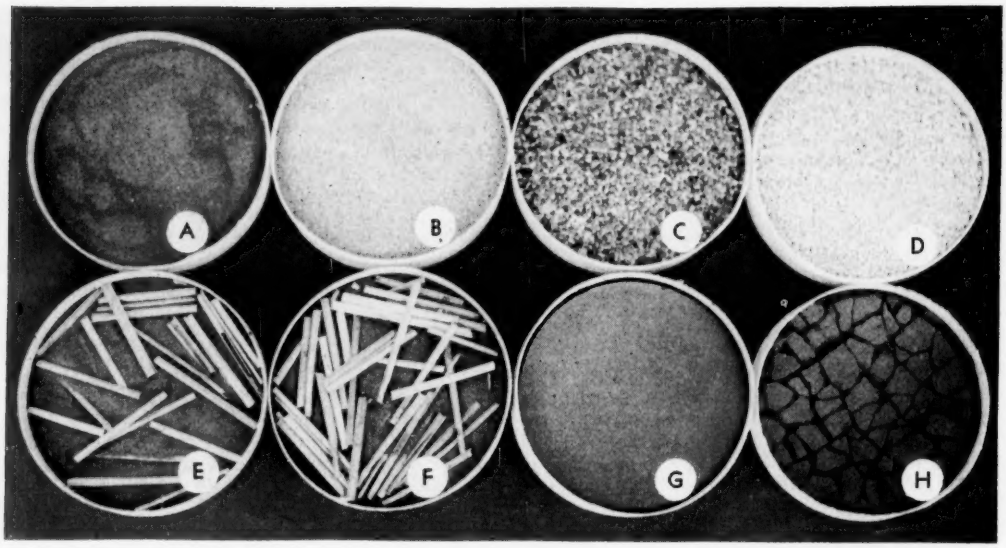

FIG. 1.—Oviposition dishes containing several test media. A, soil; B, fine sand; C, coarse sand; D, perlite; E, soil+20 straws; F, soil+40 straws; G, solid surface; H, cracked surface.

dissolved in the minimum amount of hot water needed to wet the media, blended thoroughly, and oven dried. This method resulted in very uniform and intense colors.

The number of moths used per test varied with the number of dishes of media used in the cage, but averaged four moths per dish. A honey-water mixture was provided as food in all tests. Moths were permitted to oviposit over a 2- to 5-day period after which media were removed and eggs counted. Most tests were repeated. Dead moths were removed and counted and additional moths added before repeating a test. At the conclusion of each test any living moths were killed and all were sexed. Male:female sex ratio varied from 2.6:1.0 to 0.6:1.0 and averaged 1.3:1.0 over all tests.

Eggs from each dish were spread evenly over a cross-hatched paper and counted under a dissecting microscope at 10×. A grid in the eyepiece further facilitated counting. If there were more than 400 eggs in a dish, the total was estimated by counting only a few randomly chosen squares. Eggs were easily retained by a 60-mesh screen but either passed through or were held in the openings of a 40-mesh screen. In tests in which particles coarser than 40-mesh were used, containers were held until the eggs hatched and first instar larvae were counted. This proved necessary as eggs adhered to the large particles and could not be sifted loose. Viability of eggs was close to 100% in all tests.

Since variance of treatments within a test increased proportionately to the means, all data were transformed by log (x) for analysis. In these tests certain dishes, if containing an acceptable medium, seemed to receive an undue proportion of the total eggs. For example, in Test 8 (table 3) the soil with brown straw received 8,653 eggs but 4,140 of these were deposited in only one of the eight replicates. The logarithmic transformation minimized the weight given these large counts due solely to position within a cage while giving more weight to those treatments consistently receiving moderate numbers of eggs regardless of position. In several tests new randomizations were made before repeating the test. Both actual egg counts and transformed means are given in the tables of results. Duncan's Multiple Range Test was used to determine significance among the transformed means. Any two means not connected by the same vertical line are significantly different at the 5% level.

RESULTS.—*Soil Texture and Depth.*—Fine and coarse sand and soil were mixed in various proportions as listed in Test 1, table 1. Significantly fewer eggs were laid in soil than any other media in this test. In Test 2, fine sand was also preferred over soil which in turn received significantly more eggs than fine perlite. The coarse perlite and

Table 1.—Oviposition response of the army cutworm to differences in soil texture.

TEST	COMPARISON	NUMBER OF EGGS	
		Total	Transformed Mean
1	Fine sand	17,955	2.740
	3 fine sand:1 coarse sand	14,045	2.722
	3 fine sand:1 soil	12,430	2.673
	2 fine sand:2 soil	11,595	2.672
	1 fine sand:3 soil	10,320	2.648
	1 fine sand:2 soil:1 coarse sand	11,910	2.637
	3 soil:1 coarse sand	9,505	2.561
	2 fine sand:1 soil:1 coarse sand	9,055	2.330
	Soil	3,612	2.027
2	Fine sand	33,411	3.051
	Soil	6,433	2.437
	Fine perlite	3,247	1.756
	Coarse sand	30	0.234
	Coarse perlite	1	0.000

sand appeared unacceptable in the presence of other media. The fine perlite was too light in density to support a moth and those observed in this media were usually struggling to crawl through it.

The preference for fine sand over soil appeared due to a requirement for a solid object on which to place eggs. Nearly all eggs laid in the soil were attached to the sides of containers at a uniform depth of $\frac{1}{4}$ to $\frac{3}{8}$ inch below the soil surface (fig. 2). In media containing some sand, eggs appeared randomly distributed throughout the dishes. Since coarse sand or perlite received few eggs it appeared that a combination of loose surface over a solid substrate might be essential. Presumably fine sand provided enough solidity to form a solid oviposition substrate but maintained a sufficiently loose surface which the moth could probe with its abdomen.

The optimum depth of this loose surface was further investigated and results of these tests are given in table 2. There was no significant difference between number of eggs laid in $\frac{1}{8}$ or $\frac{3}{8}$ inch of fine sand over a solid substrate in Test 3, but the actual number of eggs laid was considerably higher when the solid substrate was only $\frac{1}{8}$ inch below the sand surface. Likewise there was no significant difference between these depths of silt over a solid substrate although the deeper depth of soil received more than twice as many actual eggs. Sand received more eggs than soil.

Although not significant by the tests made, further tests and observations indicated that these probably represented true biological differences. In all tests involving loose surfaces it was possible for moths to work this surface about, invariably towards the edge of the dish, thus creating a variable depth. Observations indicated that under these conditions most of the eggs in sand were near the center of the dish where shallower depths prevailed while in soil eggs were placed nearer the edge at deeper depths.

Solid surfaces without a loose covering were refused in all tests. Results of Test 4 indicate that cracks in an otherwise solid surface were also refused, but that either a solid or cracked substrate beneath a loose surface was acceptable. Cracks in the substrate may influence the depth of loose surface necessary and further confirmed previous observations that a specific depth was needed. Results of Test 5 indicated that a shallower loose surface was acceptable over a cracked substrate. The number of eggs laid did not differ significantly between $\frac{3}{8}$ and $\frac{1}{16}$ inch loose soil over a cracked substrate. However, there was a difference in placement of these eggs. Figure 3 shows eggs placed in the cracks under $\frac{1}{16}$ inch of soil but on the substrate surface when the overlying layer of loose soil was increased to $\frac{3}{8}$ inch. Test 6 gave similar results. There was no significant difference between $\frac{1}{16}$ and $\frac{3}{8}$ inch depths of loose soil over a cracked substrate but the greater depth resulted in more eggs being deposited over a solid substrate.

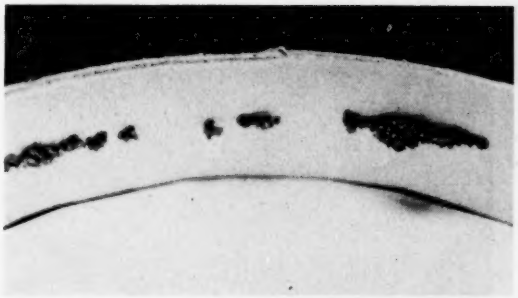

FIG. 2.—Dish with soil removed showing eggs attached at uniform depth to side of container.

Filling only the cracks with loose soil did not result in an attractive media in Test 6, although a few eggs were laid. Small clods mixed with loose soil in this test provided a satisfactory oviposition media. This treatment was similar to conditions normally present in small grain fields during the egg-laying period.

Buried straw furnished a suitable substrate in Test 7 (table 3). The increase in number of eggs laid in dishes containing buried straw was not significantly greater than for soil alone, but the placement of eggs on these straws (fig. 4) rather than around the edge of the dish was convincing evidence that there was a biological difference.

Placement of straw on the soil surface in Test 7 did result in a significant increase in the number of eggs deposited. This was somewhat surprising as the increased stability of the surface due to straws would, if anything, be expected to interfere with oviposition. However, the addition of straw added another variable, color, which appeared of importance.

Mulch and Color.—As seen in Test 7, the addition of straw on the surface increased the number of eggs laid in soil. Further results are also given in table 3. Test 8 showed undyed or yellow straws increasing the number of eggs laid in soil over the number laid when a brown or green straw mulch was used. Test 9, using two rates—20 and 40 straws per dish—showed this same color response. Soil without straw ranked intermediate in the number of eggs laid in both tests.

The addition of colored straws to the lighter colored

Table 2.—Oviposition response of the army cutworm to different depths of loose surface and types of substrate.

TEST	COMPARISON	NUMBER OF EGGS	
		Total	Transformed Mean
3	$\frac{1}{8}$″ sand over solid substrate	12,085	2.702
	$\frac{3}{8}$″ sand over solid substrate	6,795	2.484
	$\frac{3}{8}$″ soil over solid substrate	3,430	2.192
	$\frac{1}{8}$″ soil over solid substrate	1,375	1.841
4	$\frac{3}{8}$″ soil over cracked substrate	22,100	3.229
	$\frac{3}{8}$″ soil over solid substrate	18,560	3.134
	Cracked soil surface	50	0.287
	Solid soil surface	15	0.133
5	$\frac{3}{8}$″ soil over solid substrate	5,245	2.606
	$\frac{3}{8}$″ soil over cracked substrate	4,150	2.339
	$\frac{1}{16}$″ soil over cracked substrate	3,960	2.334
	$\frac{1}{16}$″ soil over solid substrate	2,665	2.122
6	$\frac{3}{8}$″ soil over solid substrate	1,525	2.190
	Clods mixed with soil	790	1.997
	$\frac{1}{16}$″ soil over solid substrate	675	1.930
	Soil in surface cracks	150	1.347

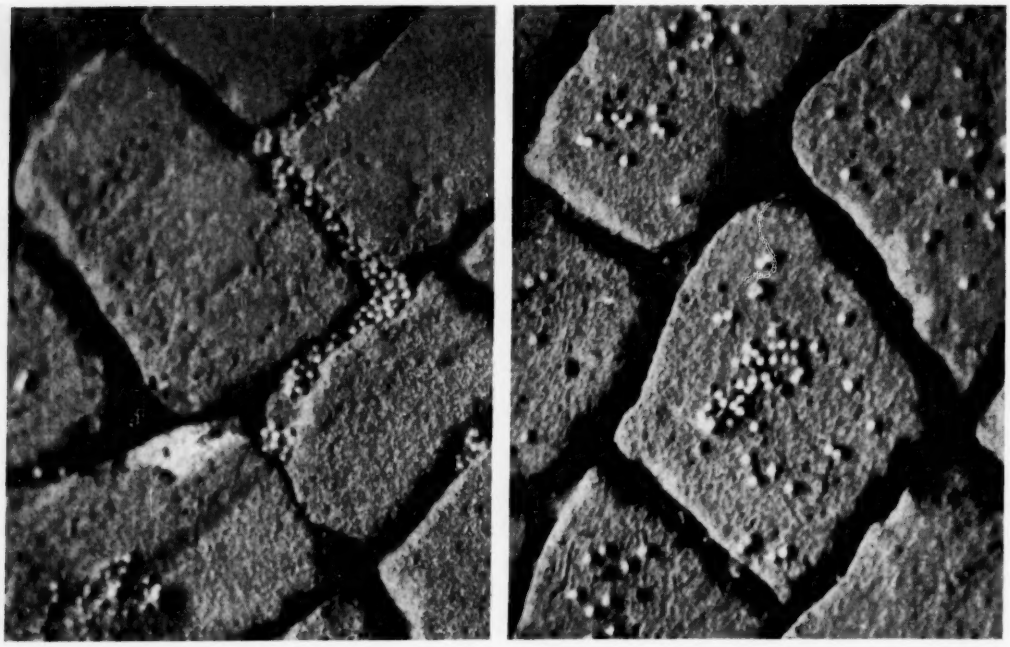

FIG. 3.—Loose soil removed showing placement of eggs in cracks (left) beneath 1/16″ of soil but on substrate top (right) beneath ⅜″ soil.

sand appeared of less importance. In Test 10 the addition of 40 green straws resulted in the fewest eggs, but other differences were not significant.

In Test 11 (table 4) 20 to 40 green or yellow straws were added to dishes of sand which were wetted and allowed to dry, forming a crusted surface. In this test, bare sand received more eggs than sand to which any color or amount of straw had been added. There was no significant difference between treatments, but a compar-

FIG. 4.—Top ⅜″ soil removed showing placement of eggs on straw buried beneath surface.

Table 3.—Oviposition response of the army cutworm to differences in straw mulch.

TEST	COMPARISON	NUMBER OF EGGS	
		Total	Transformed Mean
7	Soil+40 surface straws	8,220	2.574
	Soil+20 surface straws	7,605	2.535
	Soil+40 buried straws	6,880	2.352
	Soil+20 buried straws	4,285	2.055
	Soil alone	3,055	2.049
8	Soil+yellow straw	9,320	3.750
	Soil+undyed straw	7,528	2.750
	Soil alone	5,065	2.438
	Soil+green straw	5,765	2.351
	Soil+brown straw	8,653	2.203
9	Soil+40 yellow straws	11,145	2.961
	Soil+20 yellow straws	10,300	2.871
	Soil alone	5,536	2.568
	Soil+20 green straws	5,112	2.440
	Soil+40 green straws	3,677	2.349
10	Sand+40 yellow straws	4,430	2.565
	Sand+20 yellow straws	7,015	2.560
	Sand alone	6,985	2.533
	Sand+20 green straws	5,815	2.511
	Sand+40 green straws	3,215	2.197

ison of straw vs. none was significant. In this test straws stabilized the surface, which made it less easily broken by the moths than bare sand. Results in this test were extremely variable with eggs being concentrated in the few dishes in which the crusted surface was broken.

The addition of straws to dry sand or soil, at least up to 40 per dish, did not seem to interfere with oviposition. Test 12 compared green and yellow sand with and without 40 straws of the same color as the sand. In this case, the only difference was between colors, with straws having no apparent effect.

Since light intensity was not controlled in any test, it is impossible to say whether there was any spectral response as Callahan (1957) showed to exist for the corn earworm, *Heliothis zea* (Boddie). It was observed in all tests that those media reflecting the more light received the most eggs. This would make it appear that light intensity was involved. Even in those tests in which there was no color difference between media, it was noted that most eggs were laid in those replicates nearer the window. Egg laying was never begun before 7 p.m. and often not until 9 p.m. in these tests. However, on moonlight nights a visual difference between replicates as well as treatments could be noted and it seemed possible that moths were more attracted to the lighter surfaces. This might also explain why the light-colored sand received more eggs than soil in all tests even though both media, when of proper depths over a solid substrate, provided suitable oviposition sites.

DISCUSSION.—Moths of the army cutworm respond ovipositionally both to visual and physical differences in soils. It appears that any potential oviposition site must be visually attractive in relation to adjacent sites. If other soil properties are similar, the most reflective surface received the most eggs. It must be emphasized that a loose surface and solid substrate were essential regardless of visual attractiveness and that moths confined in cages responded ovipositionally to very small differences in soil texture or depth of loose surface.

Under field conditions, visual attractiveness may be of greatest importance. Soil conditions fulfilling the other physical requirements for egg laying are present in most cultivated fields during the oviposition period. The importance of visual attractiveness could well explain the preference for barley over wheat under otherwise identical cultural practices since, at the time moths are ovipositing, winter barley is considerably lighter in color.

The physiological mechanisms in the moth responsible for this selection are unknown. In addition to the apparent light response, the moth requires a loose surface, which it probes with its abdomen, and a solid object on which the eggs are laid.

Table 4.—Oviposition response of the army cutworm to physical and visual differences in sand created by addition of straw mulch.

TEST	COMPARISON	NUMBER OF EGGS	
		Total	Transformed Mean
11	Crusted sand	14,720	1.875
	Crusted sand+20 green straws	3,810	1.815
	Crusted sand+20 yellow straws	4,527	1.794
	Crusted sand+40 green straws	6,990	1.760
	Crusted sand+40 yellow straws	2,525	1.731
12	Yellow sand	8,420	2.512
	Yellow sand+yellow straw	6,875	2.394
	Green sand	4,270	2.228
	Green sand+green straw	4,735	2.212

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Ruelene Administered Free-choice in a Mineral Mixture for Cattle Grub Control[1]

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ABSTRACT

Ruelene® (4-*tert*-butyl-2-chlorophenyl methyl methylphosphoramidate) incorporated in a salt-bonemeal mixture (3 to 1 by weight) was offered, at two levels on a free-access basis, to calves for the control of cattle grubs, *Hypoderma lineatum* (De Vill.) and *H. bovis* (L.). Ruelene quantities of 8 and 24 grams per pound of the mineral mixture were each offered to a lot of eight calves as their only source of mineral supplement for 62 days beginning in December, 1959. All grubs were killed in the calves in the treated lots while the control lot of calves averaged 25.4 grubs each. No symptoms of toxicity were noticed. Erythrocyte cholinesterase activity was depressed significantly in both treated groups. Ruelene at the higher level reduced palatability of the mixture.

Free-choice administration of a chemical that has systemic action against cattle grubs, *Hypoderma bovis* (L.) and *H. lineatum* (De Vill.) has been shown to be an effective method of treating cattle for grubs under dry-lot feeding conditions. Successful cattle grub control has been demonstrated by incorporating ronnel in a 3:1 salt-bonemeal mixture and making it available on a free-choice basis to calves fed in dry-lot (Rogoff & Kohler 1959). These authors also discussed limitations of this method of treatment and reported toxicity in calves at one of the levels used. The administration of several organophosphates at various levels as feed additives in the grain ration of cattle has also been shown by Sturdy (1958a, 1958b), French *et al.* (1958), DeFoliart *et al.* (1958), Kohler *et al.* (1959), Weintraub *et al.* (1959), and Rich & Ireland (1959) to be an effective treatment for grubs.

Cattle almost invariably are fed salt as a supplement whether they are kept on the range, in small pastures or in dry-lot. This mode of treatment for grubs, with the insecticide in salt or a mineral mixture, would appear to merit wide spread acceptance if a uniform dosage among animals could be achieved.

MATERIALS AND METHODS.—Ruelene® (4-*tert*-butyl-2-chlorophenyl methyl methylphosphoramidate), furnished by the Dow Chemical Company, was used as the test chemical and mixed in a salt-bonemeal (3 parts salt and 1 part bonemeal by weight) mixture. Procedures used in this study were comparable to those used by Rogoff & Kohler (1959). Ruelene was used at levels of 8 and 24 grams per pound of the salt-bonemeal mixture. The animals were presumably grubby steer calves from central South Dakota and were allotted on a basis of weight. The treated mineral mixtures were each offered to a lot of eight calves as their only source of mineral supplement on December 17, 1959, and continued for 62 days. The ration fed to all calves consisted of 4 pounds of corn and oats per head daily and brome hay fed free-choice. Grub extractions were made at approximately monthly intervals for 3 months, beginning February 5, 1960.

A semimonthly weigh-back of the Ruelene-mineral mixture permitted the calculation of the average consumption of Ruelene at these intervals during the treatment period. Two other grub treatments and a control lot were held in adjacent lots concurrently with this test (Rogoff & Kohler 1960). These treatments had eight animals each but were housed in two lots of 12 animals. A similar salt-bonemeal mixture without Ruelene was offered these lots on a free-choice basis and consumption recorded as a comparison with any effect the Ruelene may have on palatability of the mixture. Salt-bonemeal consumption was recorded on all lots for a 67-day period after the Ruelene treatment was suspended in the two treated lots. A pretreatment blood sample was obtained from each calf on December 10, 1959, and two post-treatment blood samples were taken on the 23rd and 64th days for erythrocyte cholinesterase determinations. A method slightly modified from that of Michel (1949) was used for the erythrocyte cholinesterase determinations.

Table 1.—Consumption, grub infestation and erythrocyte cholinesterase activity of calves allowed free-access to a Ruelene-mineral mixture for 62 days.

	TREATMENT (GRAMS OF RUELENE/LB. OF MIX)		
	24	8	0
Total calves (Dec. 17, 1959)	8	8	8
Consumption (per calf)			
Total mixture (oz./day)	0.31	0.71	0.66–1.00[a]
Ruelene (mg.)			
Total (per kg., median)	170	126	—
Per day (per kg., initial)	2.75	2.03	—
Infestation			
Calves infested	0	0	6
Grubs/calf (range)	0	0	0–57
Grubs/calf (avg.)	0	0	25.4
Per cent reduction	100	100	0
Erythrocyte cholinesterase Jan. 7, 1960 (23rd day after treatment)			
ΔpH units/hour			
Range	0.15–0.42	0.24–0.49	0.30–0.59
Average	0.30	0.37	0.45
Activity (% initial)	64[b]	76	92
Erythrocyte cholinesterase Feb. 17, 1960 (64th day after treatment)			
ΔpH units/hour			
Range	0.20–0.39	0.24–0.49	0.36–0.66
Average	0.24	0.33	0.54
Activity (% initial)	51[c]	67[c]	110

[a] Consumption in each of 2 check lots.
[b] Significant at 5% level.
[c] Significant at 1% level

RESULTS.—Ruelene consumption, grub reduction and erythrocyte cholinesterase activity of the calves are shown in table 1. Grub reduction of 100% for each of the two levels of Ruelene offered in the mineral mixture was obtained. Seventy-five per cent of the calves in the control lot were infested with an average of 25.4 grubs per calf.

More than twice as much total mineral was consumed by the calves receiving the lower level of Ruelene (0.71 and 0.31 ounces per calf per day). The difference in consump-

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[2] Associate Animal Husbandman and Entomologist, respectively, in the South Dakota Agricultural Experiment Station.

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tion of Ruelene between the two levels, however, averaged only 0.72 mg./kg. per calf per day. The lower average consumption of the Ruelene-mineral mixture at the higher level suggests that Ruelene at the level of 24 grams per pound of mineral reduced the palatability of the mixture considerably. A comparison of the mineral consumption of the two control lots over the same period (0.66 and 1.0 ounce of total mineral per calf per day) indicates that the mixture containing 8 grams per pound did not decrease the palatability in all cases.

Mineral consumption was calculated on all lots after Ruelene was removed from the treated lots in a subsequent period of 67 days. In this period the lot which received the lower level consumed the most mineral (1.2 ounces/calf/day) while the lot receiving the higher level consumed 0.83 ounce/calf/day. The lot of calves that had previously received Ruelene at the higher level ate less mineral than the others, a variability that is not readily explainable. The control lots each averaged 0.77 ounce of mineral per calf per day in this post-treatment period. The variability of mineral consumption between lots, and very possibly between calves within a lot fed on a similar ration, may be a disadvantage of a free-choice mineral method of treatment of cattle for grubs. In this test, however, all the grubs in each Ruelene-treated lot were apparently killed in their migrating stages. No symptoms of toxicity were observed in either treated lot.

Erythrocyte cholinesterase activity at the 23rd day post-treatment, in per cent of pretreatment levels, was 76, 64 and 92 for the 8 gram/lb., 24 gram/lb. and control lots, respectively. This depression continued to the 64th day post-treatment, which in per cent of pretreatment was 67, 51 and 110, respectively. An analysis of variance showed no significant differences at the 23rd day post-treatment between the two levels of Ruelene or between the control lot and the lot receiving the low level of Ruelene. A similar statistical study revealed no differences between the two treated lots at the 64-day post-treatment period but significances at the 1% level between each treated lot and the control. The ranges of cholinesterase inhibition in the treated lots suggest that most of the animals were receiving some of the organophosphate but that an overlapping occurred in each of the treated lots with the control lot. The variability of cholinesterase activity in these data does not indicate the precise Ruelene intake of each of the treated animals.

Treatment of cattle with an insecticide for the control of cattle grubs by means of a salt or mineral mixture would be labor-saving, and a simple method of application. More study is needed with chemicals that are effective against grubs and that are suitable for a free-choice, oral dosing; possible ways of controlling intake and uniformity of consumption in cattle on various rations; and timing and length of the free-access period or periods. At the levels of Ruelene used in our tests in the salt-bonemeal mixture, no obvious signs of toxicity were noticed and all the grubs were killed, indicating that more study may be in order with Ruelene with various rations and with cattle under various management practices.

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Effect of Various Insecticide Treatments on Populations of the Green Peach Aphid on Peppers in Southern California[1]

H. H. SHOREY[2]

ABSTRACT

Various insecticides were evaluated for effectiveness against populations of the green peach aphid, *Myzus persicae* (Sulz.), on peppers during 1959–60. Most organic phosphate insecticides were relatively ineffective or caused a resurgence in aphid numbers after a fair initial control. Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide) as a dust or spray, Bayer 30911 (methyl-*O*-methyl *O*-2,4-dichlorophenyl phosphonothioate) as a dust, and dimethoate, phosphamidon, and General Chemical 4072 (diethyl-1-(2,4-dichlorophenyl)-2-chlorovinyl phosphate) as sprays appeared to be highly effective against the aphid and reduced populations to low levels for periods ranging from 4 to 20 days.

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[2] The author expresses appreciation to R. L. Hale and H. Nakakihara for their assistance with this work.

The green peach aphid, *Myzus persicae* (Sulz.), is one of the major insect pests of truck crops in southern California. On peppers the green peach aphid has attracted the attention of entomologists and growers because of its role as a vector of several serious disease-causing viruses. These viruses frequently infect large numbers of plants and cause a considerable reduction in the yield of marketable peppers. In addition, copious secretions of honeydew, which are produced by high populations of the green peach aphid, tend to increase the difficulties involved in the cleaning of the fruit prior to marketing.

Until recent years, the green peach aphid has been adequately controlled on peppers in southern California by the use of various organic phosphate insecticides. Recently, however, an increase in the tolerance of the aphid populations to malathion, parathion, Diazinon, and Phosdrin has become apparent. A similar difficulty has been noted by Wolfenbarger (1960), who observed that several materials that had been effective against the green peach aphid on potatoes in south Florida gave unsatisfactory control after several years. Shirck (1960) demonstrated that strains of the aphid from California and Washington were more tolerant to malathion than were strains from Nebraska, Illinois and Maryland.

Commercial pepper growers in many areas of southern California apply periodic treatments of DDT for control of the pepper weevil, *Anthonomus eugenii* Cano. These treatments often intensify the aphid problem, probably because of the high efficiency of DDT against many beneficial insects. In an attempt to reduce the aphid populations, most of the growers engage in an aggressive insecticide-application program. As the aphids become more difficult to control, insecticides are often applied at increasingly higher rates and shorter intervals; under these conditions the degree of control obtained usually is poor, and the aphid populations build up rapidly to the pretreatment levels or higher.

The purpose of the present study was to evaluate the effect of a number of insecticides upon populations of the green peach aphid, and to compare those materials as concerns initial kill and duration of effectiveness.

METHODS AND MATERIALS.—Eight field-plot experiments were initiated in Orange, San Diego, and Riverside Counties, California, during the period from October 1959 through July 1960. All insecticides used in the experiments were applied with hand equipment, because many pepper fields in southern California are inaccessible to tractor-mounted insecticide-application equipment. Dusts were applied with either a power-pack duster or a hand-operated rotary duster having a single orifice. Sprays were applied with a 3-gallon hand sprayer, which operated at a pressure of 40 p.s.i. and delivered approximately 30 gallons per acre through a D-3 solid-cone nozzle. The treatments in each experiment were arranged in four or five replicates. Plot dimensions ranged from 30 to 60 feet long by 3 to 6 rows wide.

The materials used were parathion, rotenone, dimethoate, ethion, ronnel, phosphamidon, Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidyl) thiophosphate), Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide), Sevin® (1-naphthyl *N*-methylcarbamate), Dylox® (dimethyl (2,2,2,-trichloro-1-hydroxyethyl) phosphonate), DIBROM® (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate), Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4*H*)-ylmethyl) phosphorodithioate), Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate), General Chemical 4072 (diethyl-1-(2,4-dichlorophenyl)-2-chlorovinyl phosphate), Bayer 30911 (methyl-*O*-methyl *O*-2,4-dichlorophenyl phosphonothioate), Bayer 29493 (*O,O*-dimethyl *O*-(4-methylthio-*m*-tolyl) phosphorothioate), and Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate 60% technical).

Table 1.—Effect of insecticides applied as dusts for control of the green peach aphid on peppers. Vista, California. October, 1959.

Treatment	Actual Toxicant per Acre (lb.)	Mean Number of Apterae per 100 Leaves[a,b]	
		1 Day	4 Days
Thiodan, 2%	1.2	49[b]	9[b]
	0.5	80[b]	44[bc]
Diazinon, 2%	1.0	107[b]	217[cd]
Parathion, 2%	0.8	257[c]	482[de]
Untreated		571[c]	1,217[e]

[a] 150 and 75 leaves per treatment were examined at 1 day and 4 days, respectively.

[b] Any 2 means in the same column having the same superscript letter are not significantly different as determined by Duncan's multiple range test at the 5% level. Original data were transformed to logarithms for analysis.

The efficiency of the various insecticidal treatments was evaluated by counts of the number of aphids on a specified number of leaves selected at random from plants in the central rows of each plot. Half the leaves examined were selected from near the tops and half from near the bases of the plants. The results obtained in the eight experiments are included in tables 1 through 8.

Translocation Study.—The excellent performance of Thiodan, particularly in a spray formulation, raised the question whether there was some translocation of this material through pepper leaves. A field study using caged green peach aphids was initiated in order to investigate this possibility.

A block of 16 bell pepper plants was selected in a field near San Juan Capistrano, Orange County. The upper surface, excluding the peripheral one-half inch, of one leaf on each of four plants was painted with a 0.25% Thiodan emulsion. This procedure was repeated on other

Table 2.—Effect of insecticides applied as dusts for control of the green peach aphid on peppers. Encanto, California. November, 1959.

Treatment	Actual Toxicant per Acre (lb.)	Mean Number of Apterae per 100 Leaves After Days Shown[a,b]			
		2	8	15	21
Thiodan, 3%	0.9	82[b]	121[b]	157[bc]	112[bcd]
	0.6	160[bc]	302[c]	246[cd]	150[bcd]
Diazinon, 2%	0.9	203[bc]	476[c]	239[cd]	46[bc]
Dylox, 5%	1.4	392[cd]	364[c]	491[cd]	526[cd]
Parathion, 2%	0.9	310[cd]	241[bc]	650[cd]	522[cd]
Sevin, 2%	1.0	345[cd]	577[c]	817[d]	766[d]
Untreated		685[d]	491[c]	49[b]	14[b]

[a] 100 leaves per treatment were examined on each sampling date.

[b] Same as in table 1.

Table 3.—Effect of insecticides applied as dusts for control of the green peach aphid on peppers. San Diego, California. December, 1959.

Treatment	Actual Toxicant per Acre (lb.)	Mean Number of Apterae per 100 Leaves[a,b] 3 Days	7 Days
Thiodan, 3%	2.8	3[b]	1[b]
Rotenone, 2%	1.2	136[c]	52[c]
Rotenone, 1%	0.4	264[cde]	178[cd]
Dibrom, 4%	5.0	136[cd]	215[d]
Sevin, 2%	0.8	245[cde]	327[d]
Guthion, 2%	0.6	348[de]	291[d]
Untreated		440[e]	290[cd]

[a] 100 leaves per treatment were examined on each sampling date.
[b] Same as in table 1.

Table 4.—Effect of insecticides applied as dusts for control of the green peach aphid on peppers. San Clemente, California. April, 1960.

Treatment	Actual Toxicant per Acre (lb.)	Mean Number of Aphids per 100 Leaves After[a,b] 5 Hours Alate	5 Hours Apterous	3 Days Alate	3 Days Apterous	7 Days Alate	7 Days Apterous
Thiodan, 3%	1.2	17[b]	196[b]	135[bc]	183[b]	53[b]	338[b]
Dimethoate, 2%	1.2	113[a]	738[e]	96[b]	261[bc]	50[b]	350[b]
Diazinon, 2%	0.9	79[bc]	179[b]	153[bc]	308[c]	66[b]	571[bc]
Trithion, 2%	0.8	75[bc]	542[e]	159[c]	534[d]	81[b]	775[c]
Rotenone, 2%	0.6	75[bc]	821[e]	143[bc]	601[de]	68[b]	479[bc]
Ethion, 2%	1.0	100[bc]	1083[e]	183[c]	795[de]	58[b]	581[bc]
Untreated		146[c]	1133[e]	135[bc]	920[e]	50[b]	584[bc]

[a] 24 leaves per treatment were examined after 5 hours; 80 leaves per treatment were examined after 3 and 7 days.
[b] Same as in table 1.

Table 5.—Effect of insecticides applied as dusts and sprays for control of the green peach aphid on peppers. Oasis, California. April, 1960.

Treatment	Actual Toxicant per Acre (lb.)	Mean Number of Apterae per 100 Leaves After Days Shown[a,b] 1	3	7	14	20
Dusts:						
Thiodan, 3%	1.6	56[bc]	5[b]	13[b]	19[b]	39[c]
Dimethoate, 2%	1.1	258[d]	193[cd]	428[cd]	725[e]	274[d]
Diazinon, 2%	0.8	104[c]	204[cd]	588[cd]	692[e]	172[d]
Sprays:						
Thiodan	1.4	60[bc]	7[b]	11[b]	31[b]	6[b]
Dimethoate	1.2	28[b]	25[b]	8[b]	15[b]	19[bc]
Diazinon	1.2	59[bc]	58[c]	281[c]	697[e]	248[d]
Untreated		459[d]	557[d]	989[d]	1240[e]	168[d]

[a] 160 leaves per treatment were examined on each sampling date.
[b] Same as in table 1.

plants with an 0.25% dimethoate emulsion and an 0.25% Diazinon emulsion. The remaining four plants served as untreated checks.

After the emulsion had dried, a cage containing 10 apterous green peach aphids was fastened against the treated surface of each leaf. A similar cage was placed on the lower epidermis of each leaf, opposite the treated surface. Numbers of living green peach aphids found in the cages after 3 days are listed in table 9.

DISCUSSION.—Many of the insecticides tested caused a marked resurgence in the aphid populations, often within the brief time interval of from 3 to 7 days. The following factors seem to be involved in a rapid aphid buildup following treatment with various insecticides.

1. A certain proportion of the aphid population survives the treatment because it is sheltered from direct contact with the insecticide and/or because it is relatively tolerant to the insecticide. The green peach aphid typically remains on the lower surface of pepper leaves. Many of the survivors of an insecticide application are found below the large lower leaves, which often lie almost flat upon the ground. Materials which have a marked

Table 6.—Effect of insecticides applied as sprays for control of the green peach aphid on peppers. Oasis, California. May, 1960.

Treatment	Actual Toxicant per Acre (lb.)	Mean Number of Apterae per 100 Leaves[a,b] 1 Day	4 Days	7 Days
Phosphamidon	1.1	17[b]	11[b]	53[bc]
Thiodan	0.6	75[b]	18[bc]	19[b]
Dibrom	2.2	31[b]	53[c]	103[c]
Ronnel	1.1	373[c]	419[d]	916[d]
Untreated		258[c]	203[d]	125[c]

[a] 120 leaves per treatment were examined on each sampling date.
[b] Same as in table 1.

Table 7.—Effect of insecticides applied as dusts and sprays for control of the green peach aphid on peppers. San Luis Rey, California. July, 1960.

Treatment	Actual Toxicant per Acre (lb.)	Mean Number of Apterae per 100 Leaves[a,b] 1 Day	3 Days
Dust:			
Bayer 30911, 5%	1.8	4[b]	2[b]
Sprays:			
GC—4072	0.9	12[bcd]	3[b]
Bayer 29493	0.9	13[bc]	4[b]
Phosphamidon	0.4	11[bcd]	0[b]
Thiodan	0.5	24[cde]	1[b]
Untreated		75[e]	28[c]

[a] 160 leaves per treatment were examined on each sampling date.
[b] Same as in table 1.

Table 8.—Effect of insecticides applied as dusts and sprays for control of the green peach aphid on peppers. San Juan Capistrano, California. July, 1960.

Treatment	Actual Toxicant per Acre (lb.)	Mean Number of Apterae per 100 Leaves[a,b] 3 Days	7 Days	13 Days
Dust:				
Bayer 30911, 5%	1.9	0[b]	6[bcd]	20[b]
Sprays:				
Dimethoate	0.4	1[bc]	0[b]	5[b]
Thiodan	0.5	1[bc]	3[bcd]	28[b]
GC—4072	0.9	4[bcd]	1[bc]	16[b]
Phosdrin	0.4	14[de]	19[de]	48[b]
Bayer 29493	0.9	6[cd]	53[e]	373[c]
Untreated		28[e]	20[cde]	68[b]

[a] 160 leaves per treatment were examined on each sampling date.
[b] Same as in table 1.

Table 9.—Numbers of living green peach aphids in cages placed on the upper and lower surfaces of pepper leaves painted on the upper surface with 0.25% emulsions of three insecticides.

Treatment	No. of Living Aphids After 3 Days	
	Upper Surface[a]	Lower Surface[a]
Thiodan	7[b]	53[c]
Dimethoate	2[b]	0[b]
Diazinon	0[b]	58[c]
Untreated	23[c]	57[c]

[a] Forty aphids per treatment were caged on upper surface and lower surface of leaves. Increase in numbers represents aphids produced during the 3 days.
[b] Same as in table 1.

systemic or fumigant action will reduce the number of these sheltered survivors.

2. Most of the chemicals that are used for control of the green peach aphid seem to be highly effective against the complex of beneficial insects which, if left unhindered, undoubtedly could often reduce the aphid populations to low, noneconomic levels. It is of interest to note that five of the eight fields selected for insecticide evaluation had declining populations of aphids in the untreated plots. The application of certain insecticides, notably Sevin, Dylox, parathion, and ronnel, completely reversed this trend and caused a rapid aphid buildup. In each of the five fields, signs of intense biological control activity were noted; the beneficial insects most commonly observed were coccinellid adults and larvae, syrphid larvae, larvae of the green lacewing, *Chrysopa* spp., and the hymenopterous parasite *Aphidius nigriteleus* Smith. Research now in progress indicates that Thiodan is more toxic to the green peach aphid than to some of the beneficial insects. This selectivity may partially explain the general absence of a resurgence in plots sprayed with Thiodan.

3. Under favorable environmental conditions, populations of the green peach aphid can double in numbers within 3 days (tables 1, 5, 6, and 8). Therefore, after the initial kill of susceptible, exposed aphids, a resurgence is apt to occur following treatment with an insecticide having a relatively short period of residual activity and a high efficiency against biological control agents.

Five of the insecticides evaluated gave good control of the green peach aphid on peppers and did not stimulate a rapid buildup in aphid populations. These materials were Thiodan as a dust or spray, Bayer 30911 as a dust, and dimethoate, phosphamidon, and General Chemical 4072 as sprays.

Thiodan is relatively slow in action and generally required 3 days to suppress the aphid populations to their lowest levels. Thiodan applied as a spray has been very effective at rates as low as approximately one-half pound of actual toxicant per acre (tables 6, 7 and 8). As a dust, however, an approximately 0.5-pound rate showed a tendency, which is not significant, to be less effective than a 1-pound rate (tables 1 and 2). No evidence of a translocation of Thiodan into pepper leaves was found (table 9). The increase in numbers of aphids caged for 3 days on the undersides of leaves treated on the upper surface with Thiodan and Diazinon indicated the ability of those aphids to produce viable offspring. The high efficiency of Thiodan has been demonstrated by Schopp & Landis (1959) to be attributable partially to a fumigant action which causes a mortality of concealed aphids.

The systemic characteristic of dimethoate and phosphamidon, causing an increased exposure of aphids feeding on the lower surfaces of the leaves by virtue of penetration of the toxicant into the leaves, may help to explain why these materials are highly effective against the green peach aphid while many other organic phosphate insecticides are relatively ineffective. This assumption is supported by the observation that dimethoate applied as a spray is much more effective than an equivalent dosage of the same material as a dust (table 5). Dimethoate carried in a liquid probably penetrates into the leaf more readily than it does from a dust particle.

In the one test conducted while a heavy alate flight was in progress (table 4), no material appeared to be effective 7 days after application. The alatae were little affected by the insecticides after 3 days and apparently continued to reinfest the plants and to produce apterae until insecticide residues had diminished to the level at which a rapid reestablishment of the apterous population could occur. A less intense flight was in progress during test No. 8 (table 8) and probably contributed to the poor control given by most materials after 13 days. Similar difficulties on potatoes treated during heavy flights of the green peach aphid have been reported by Landis & Schopp (1958).

At 2 to 4 days following application, the following materials gave fair aphid control or good control followed by a rapid aphid resurgence: parathion, dimethoate, and rotenone as dusts, Diazinon and Dibrom as dusts or sprays, and Bayer 29493 as a spray. The remaining materials tested were relatively ineffective against the green peach aphid. These were Sevin, Dylox, Guthion, ethion, and Trithion as dusts and ronnel and Phosdrin as sprays.

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Fecundity and Fertility of Two Strains of the Plum Curculio, Reciprocal Crosses and the F_1 Generations[1]

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Abstract

The fecundity and fertility of two strains of plum curculio, *Conotrachelus nenuphar* (Hbst.), the reciprocal crosses and the F_1 generations were compared under laboratory conditions. The two strains were a laboratory strain reared continuously from a culture collected from the area of Raleigh, North Carolina in 1949 and a northern strain collected in the field at Geneva, New York. The laboratory strain is characterized by continuous reproduction under laboratory conditions while the northern strain requires hibernation to initiate oviposition. The laboratory strain produced significantly more eggs than the northern strain with fertility being equal. The northern female×laboratory male cross resulted in significantly lower fertility and fecundity than the reciprocal cross. In the F_1 generations, fertility was normal with reduced fecundity in the F_1 laboratory ♀×northern ♂ cross.

It is commonly known that the plum curculio, *Conotrachelus nenuphar* (Hbsh.), has only one generation annually in the northern areas of its distribution and usually more than one generation in the southern areas. The approximate boundary between these two areas has been shown by Chapman (1938).

Stearns (1931) pointed out that the number of broods appeared to be controlled by factors other than temperature and initiated a study to determine the cause of the difference in the number of generations, but the results were not conclusive.

In attempting to rear the species in the laboratory, Smith (1957) reported that females from the area of Geneva, N. Y. would not produce eggs under laboratory conditions (80° F.) unless previously hibernated. However, with females obtained in 1949 from the area of Raleigh, North Carolina, where more than one generation occurs, a culture was selected which did not require hibernation before oviposition. This culture has been maintained continuously to date. It was thus apparent that two strains of the plum curculio exist, (1) a strain in the North which requires hibernation before oviposition and (2) a strain in the South which does not require hibernation. More recent investigations have indicated that both strains are represented in the multibrooded area (Smith, unpublished data).

It was the object of this study to compare the fecundity and fertility of both strains, reciprocal crosses of the two strains and F_1 generations.

Materials and Methods.—The rearing method developed by Smith (1957) was used for the laboratory (L) strain. Northern (N) strain adults were reared in an insectary from naturally infested fruits collected from unsprayed trees in the area of Geneva, New York. F_1 generation adults were reared in the laboratory by the method cited.

Hibernation requirements for the northern females were fulfilled by exposure to outdoor temperature in October and November. Males were hibernated with the females in tests with pairs of the northern strain and the N ♀×L ♂ cross. Males of the L ♀×N ♂ cross were not exposed to outdoor conditions.

Individual pairs were confined under jelly glasses supported on screen racks. Shortly after oviposition began the males were removed. For the F_1 generation, groups of males and females of similar parentage were confined together until oviposition commenced and the females were then isolated for further study.

Green thinning apples provided food and sites for oviposition. The apples were removed daily if oviposition scars were visible and held in quart jars with screen tops at 80° F. Fecundity and fertility counts were made after a minimum of 3 days. Inspection of the apples was made with the aid of a binocular microscope. A flat metal probe was used to lift the apple epidermis covering the eggs. The criteria for fertility were either a hole into the apple made by the hatched larva or the head capsule or mandibles of the unhatched larva visible through the chorion.

The laboratory was maintained at a temperature of 80° F. ± 2 and 50%–70% relative humidity.

The differences in the various groups were compared for significance at the 5% level.

Results and Discussion.—The fecundity and fertility records of the various groups are shown in table 1. There was a significant difference between the average of 706 eggs laid by the laboratory strain and the 212 laid by the northern strain.

In a previous study with the same strains, Smith (1957) reported averages of 489 and 197, respectively, for females of the laboratory and northern strains. Although the northern strain average was in general agreement with that of the present study, the laboratory strain average was considerably lower than the 706 eggs reported here. It was suggested (Smith 1957) that in the course of laboratory rearing, chance selection had occurred for the most prolific females, and the results of this study suggest that a continued increase in fecundity of the laboratory strain has taken place in the two-year interval between the two studies.

The 6% difference in the fertility of the two strains was not significant.

The reciprocal crosses showed slight reductions in fecundity over the northern and laboratory strains, but the differences were not significant. The fecundity records of the crosses were significantly different from each other.

There was a highly significant decrease in the fertility of the N ♀×L ♂ cross from that of the northern and laboratory strains and the reciprocal cross. Since many eggs contained fully developed but dead embryos, it is suggested that some form of incompatibility exists between the laboratory male and the northern female.

In the F_1 generation, females of the L ♀×N ♂ cross averaged significantly fewer eggs than the female parent

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Table 1.—The fecundity and fertility of individual female plum curculios of the northern (N) and laboratory (L) strains, reciprocal crosses, and F_1 generation.

					Reciprocal Crosses				F_1 Generation			
	L♀×L♂		N♀×N♂		L♀×N♂		N♀×L♂		L♀×N♂		N♀×L♂	
Female No.	Total Eggs	Per Cent Fertility	Total Eggs	Per Cent Fertility	Total Eggs	Per Cent Fertility	Total Eggs	Per Cent Fertility	Total Eggs	Per Cent Fertility	Total Eggs	Per Cent Fertility
1	1,191	97	326	91	773	92	364	32	487	95	1,137	90
2	1,179	97	311	99	678	92	349	24	485	82	861	86
3	1,062	88	306	98	618	89	289	30	375	98	676	93
4	1,042	98	303	100	552	90	285	36	353	97	625	94
5	981	91	281	96	474	66	232	28	325	98	605	95
6	941	47	269	100	102	61	185	17	304	95	595	91
7	933	98	262	98			184	11	295	99	575	87
8	910	97	235	98			177	10	164	98	510	93
9	836	86	228	99			156	21	121	100	483	99
10	736	98	221	92			136	39	90	91	414	96
11	668	99	218	99			130	25	50	100	362	96
12	566	96	207	99			108	41	39	95	354	59
13	551	96	197	98			95	6	25	80	305	93
14	535	95	175	100			83	23			83	54
15	508	95	175	95			81	64			80	91
16	436	79	173	88			68	44			72	97
17	385	91	117	100			54	18			41	100
18	341	97	110	100			18	11			40	98
19	223	58	90	34			—	—			30	100
20	—	—	37	92			—	—			14	79
Average	706	90	212	96	533	86	166	27	239	95	393	90

Summary of statistical analyses

Crosses	Total Eggs	Per Cent Fertility	Crosses	Total Eggs	Per Cent Fertility
L♀×L♂	706	90	N♀×L♂	166[a]	27[a]
N♀×N♂	212[a]	96	L♀×N♂	533	86
L♀×L♂	706	90	F_1 L♀×N♂	239[a]	95
L♀×N♂	533	86	L♀×N♂	533	86
N♀×N♂	212	96[a]	F_1 N♀×L♂	393[a]	90[a]
N♀×L♂	166	27	N♀×L♂	166	27

[a] Difference significant at the 5% level.

strain. By contrast F_1 females of the N♀×L♂ cross produced a significantly greater number of eggs than the female parent strain. Thus in both F_1 generations, the fecundity characteristic appeared to be influenced by the male parent.

The fertility of both F_1 generations was not significantly different from that of either of the parent strains.

These preliminary results provide several interesting leads for further study. Of particular interest is the marked reduction in fertility and fecundity in the N♀×L♂ cross. Under these experimental conditions, the sexes were separated when oviposition commenced. Later matings might have resulted in higher fertility although normal fertility resulted in other crosses handled in the same manner. This incompatibility was not apparent in the F_1 generation mated with males of similar parentage.

The possibility of reducing the biotic potential of the species in nature through the release of selected strains is deserving of further study. Such a possibility is particularly appealing in the case of this species which is long-lived, adaptable to mass laboratory rearing and occurs at relatively low population levels per unit area.

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Diapause in the Sugarcane Borer, *Diatraea saccharalis*[1]

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Abstract

Studies of diapause in larvae of the sugarcane borer, *Diatraea saccharalis* (F.), were conducted by periodically collecting mature or nearly mature larvae from a sugarcane field near Port Allen, Louisiana from July 22, 1959 until March 22, 1960. These larvae were reared on pieces of fresh corn stems in a dark cabinet at a constant temperature of 80° F. Those living longer than 19 days in the laboratory before pupation or death were considered in diapause. This arbitrary period of 19 days was the upper 95% fiducial limit for the time required for pupation by fourth instar larvae reared from eggs in the laboratory at 80° F.

It was concluded that a facultative diapause is probably present among full grown sugarcane borer larvae in Louisiana sugarcane fields during the fall months of each year. Diapause larvae were most abundant in a field in October, November and December, 1959. Diapause development apparently required about 3 months. Larvae in diapause were not completely inactive. Diapause larvae usually did not pupate in the laboratory unless some diapause development had occurred in the field.

Studies of the biology and life history of the sugarcane borer, *Diatraea saccharalis* (F.), have been conducted by Morgan (1891), Stubbs & Morgan (1902), Holloway & Loftin (1919), Holloway *et al.* (1928), and Meadows (1938). Stubbs & Morgan (1902) and Holloway *et al.* (1928) reported that larvae lived for unusually long periods of time without food during the fall and winter months before pupating. However, diapause was not mentioned by any of these workers.

Simmonds' (1948) definition of diapause appears to be a good one. He defines diapause as a state ". . . in which a reduction of growth processes or maturation occurs which is not necessarily caused by immediate environmental influences, does not depend for its continuance on unsuitable conditions, and is not easily or quickly altered by change to a more favorable environment. However, once the state of diapause comes to an end normal growth and development are resumed." According to Steinberg & Kamensky (1936) diapause is of the "facultative" type when there are one or several generations in which few or no individuals enter diapause followed by a generation in which most or all individuals enter diapause.

The possibility of a facultative diapause among larvae of *D. saccharalis* in nature has been suggested by observations from several sources. The observations of Stubbs & Morgan (1902) and Holloway *et al.* (1928) suggest that the borers which they observed were probably either in a state of quiescence or in diapause. Long *et al.* (1958) found pupae in cane fields in relative abundance during September, 1957, but observed that little or no pupation occurred after September in spite of the occurrence of several warm days in October. The pinacular spots, plainly visible on most surgarcane borer larvae during the spring and summer, typically become much lighter in color or are completely lost during the fall and winter months. Also, during these months many larvae in nature appear to be unusually yellow in color. Similar color changes have been reported in the closely related species, *Diatraea lineolata* Walk. and *Zeadiatraea grandiosella* (Dyar), by Kevan (1944) and Hensley (1960), respectively, and are now generally considered to be associated with diapause in these insects.

The studies reported in this paper were conducted to determine whether overwintering *D. saccharalis* larvae normally go into diapause, or merely quiescence, and to describe the seasonal incidence of diapause in case it was found.

Methods and Materials.—Sugarcane borer larvae were collected periodically from a field of sugarcane (variety C.P. 47-193, plant cane) located on Westover Plantation near Port Allen, Louisiana. Collections were made from July 22, 1959 until March 22, 1960, usually at intervals of approximately 1 month. The cane in this field was cut for harvest November 11, 1959, and all larvae collected after this date were found in cane tops and pieces of stalks left in the field after the harvesting operations.

The presence of diapause in mature or nearly mature field-collected larvae was determined by rearing these larvae in a dark cabinet at a constant temperature of 80° F. They were reared separately in cotton-stoppered glass vials 20 mm. wide and 80 mm. tall on tender pieces of young corn stems. The corn plants were grown in the field from Louisiana-521 seed corn during the spring and summer, and in the greenhouse during the fall and winter. Corn plants usually had not tasseled and were from 5 to 8 weeks old when cut for feeding.

Field-collected larvae living more than 19 days in the laboratory before pupation or death were considered in diapause. Larvae reared from eggs at 80° F. on corn leaves and stems in the laboratory during March and May, 1959 required an average of 12.7 days to pupate after the third moult, and the upper 95% fiducial limit for this period was 18.6 days (Katiyar 1960). Since most field-collected larvae were at least fifth instar size, the 19-day limit is considered to give a conservative estimate of the numbers of larvae in diapause.

Results.—Table 1 shows the numbers of insects, including larvae and pupae, which were collected on each date and the percentage of each collection comprised of pupae. Pupae were relatively abundant in July, 1959, but pupation had apparently decreased considerably by late September. Only one pupa out of 645 borers collected was found during October. No pupae were found after October, although periodic collections were made until March 22, 1960.

Table 2 presents a summary of the records kept on field-collected sugarcane borer larvae reared in the laboratory at 80° F. The time required for pupation of field-collected larvae in the laboratory was relatively longer

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Table 1.—Seasonal occurrence of *Diatraea saccharalis* pupae in a sugarcane field near Port Allen, Louisiana, 1959–1960.

Dates of Collection	Number of Immature Stages Collected[a]	Per Cent of Collection Composed of Pupae
July 22, 1959	250	40.0
September 23, 1959	98	18.4
October 24, 1959	163	0.0
October 28, 1959	482	0.2
November 26, 1959	58	0.0
December 22, 1959	21	0.0
January 21, 1960	49	0.0
February 16, 1960	62	0.0
March 22, 1960	21	0.0

[a] Mostly fifth or sixth instar larvae and never smaller than fourth instar.

during October, November and December, 1959 tahn during previous or succeeding months. Averages of from 8 to 12 days were required for pupation in the laboratory prior to October and after December, 1959. The average periods of time required for pupation of field-collected larvae in the laboratory during October, November and December ranged from almost 21 to 25 days.

The percentage of field-collected larvae considered to be in diapause increased steadily from 5% in July to 67% in October, 1959 (table 2). The proportion of the population in diapause continued to be high (63% to 71%) until after December, 1959. On January 21, 1960 it was determined that 19% of the field-collected larvae were in diapause, and by March 22 the percentage of larvae in diapause was down again to 5%.

Data in table 2 also indicate the percentages of diapause larvae that were able to complete diapause development and eventually pupated in the laboratory. Sixty per cent of the diapause larvae collected from the field during December completed diapause development and pupated in the laboratory. Only 12% of the diapause larvae from the September collection pupated in the laboratory, while 40% of the larvae collected at this time were considered to be in diapause. It is also apparent from data in table 2 that the proportion of all field-collected larvae that pupated in the laboratory at different times of year was inversely related to the incidence of diapause.

Records of moulting were kept for all larvae reared in the laboratory and head capsule measurements were made whenever possible. Fifty-two per cent and 46% of all nondiapause larvae pupated at the first and second moult, respectively, after being brought to the laboratory from the field. Fifty-two per cent and 32% of the diapause larvae that pupated in the laboratory did so at the second and third moult, respectively, after they were brought from the field. Although diapause larvae usually moulted a larger number of times before pupating than nondiapause larvae, head capsule measurements showed that this supernumerary moulting was not accompanied by growth in the diapause insects.

Discussion and Conclusions.—Records given in table 1 show that pupae were not found in the field from November, 1959 through most of March, 1960. The mean temperature for October, 1959 at Baton Rouge, Louisiana was 71.6° F. (U. S. Weather Bureau, 1959). This temperature is well above the threshold for pupation in *D. saccharalis*. Nevertheless, 644 larvae and only 1 pupa were found in the field during this same month less than 10 miles from Baton Rouge (table 1). The sugarcane from which these borers were collected was green and succulent and appeared to constitute a satisfactory food supply at this time. If diapause was not present during October, 1959, it seems that more pupation than was apparent should have occurred during this month. Miscellaneous observations from other fields and other years indicate that some pupae may be found during early and middle winter but that these are relatively rare until late winter or early spring in Louisiana.

Data presented in table 2 show that the earliest indication of an increase in the incidence of diapause in a sugarcane field was in August, 1959. The percentage of insects in diapause increased rapidly in September, and was apparently at a maximum during October, November and December, 1959. Although these data indicate that between 63% and 71% of the field population was in diapause during October, November and December, it is well to recall that the criteria used here for determining diapause were arbitrary.

The fact that 40% of the larvae collected in September were considered in diapause and that only 12% of these larvae pupated indicates that complete diapause development usually was not accomplished in the laboratory. However, larvae collected on successively later dates during the months of maximum occurrence of diapause in the field showed an increasing tendency to pupate under laboratory conditions (table 2). Apparently it was necessary for some diapause development to be completed in the field before the remainder could be completed in the laboratory.

The criterion used for determining diapause in these studies is based upon a slower rate of development among diapause than nondiapause larvae when both types are reared under similar conditions satisfactory for rapid development. Since field-collected sugarcane borer larvae were reared in the laboratory on corn stems from plants grown in the greenhouse during the fall and winter and in the field during spring and summer, there may be reason to suspect that differences in rates of development could be caused by differences in the food plants that were grown under different conditions at different times of the

Table 2.—Pupation, longevity and diapause among larvae of *Diatraea saccharalis* collected from a sugarcane field near Port Allen, Louisiana and reared in the laboratory at Baton Rouge, Louisiana, 1959–1960.

Dates of Collection	Number of Larvae Reared	Per Cent Pupation	Days Required for Pupation in the Laboratory at 80° F.			Per Cent in Diapause	Per Cent of Diapause Larvae that Pupated
			Mean	Min.	Max.		
July 22, 1959	19[a]	94	8.6[b]	7[b]	14[b]	5	—
August 14, 1959	32[c]	83	11.1[b]	7[b]	18[b]	16	—
September 23, 1959	40	55	10.7	7	21	40	12
October 24, 1959	21	43	22.8	16	29	67	43
November 26, 1959	16	60	20.9	12	38	63	44
December 22, 1959	21	52	25.0	18	37	71	60
January 21, 1960	26	62	12.1	9	15	19	0
February 16, 1960	40	65	10.7	7	18	13	0
March 22, 1960	19	74	9.4	4	13	5	0

[a] Includes records for 1 larva that was discarded after 21 days in the laboratory.

[b] Does not include records for larvae that were discarded.

[c] Includes records for 8 larvae that were discarded after 23 days in the laboratory.

year. It is also possible that different rates of borer development resulting from differences in food grown at different times of the year could result from differences in composition of the food which in turn might be caused by the different photoperiods to which the plants were subject. However, it does not seem likely that differences in the corn plants used at different times of year could have had sufficient effect on the rate of development of the sugarcane borer in these studies to justify any serious doubts about the conclusions that are drawn concerning diapause in this insect. As far as is known to the authors diapause has never been shown to result from photoperiod acting indirectly upon an insect through the food plant.

It is not known how much moulting normally occurs among diapause sugarcane borer larvae in the field. Head capsules were occasionally found with larvae collected in the field during the fall and winter months. Sugarcane borer larvae have been reported by Holloway *et al.* (1928) to feed and moult on warm days during the winter. They have recorded as many as 14 moults in "hibernating" larvae and state that during periods of prolonged larval development ". . . the larvae continue moulting, with little or no increase, or even a decrease in size."

The presence of excrement in the rearing vials indicated that many diapause larvae did continue to feed in the laboratory. However, feeding diapause larvae did not feed as voraciously as nondiapause larvae. It appeared that diapause larvae might feed sparingly in the presence of food, but that such feeding did not always occur and was not necessary for pupation.

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Control of Aphids and Spider Mites on Strawberries with Dusts and Low- and High-Gallonage Sprays[1]

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ABSTRACT

In experiments conducted in southern California for control of the strawberry aphid (*Pentatrichopus fragaefolii* (Ckll.)) and the two-spotted spider mite (*Tetranychus telarius* (L.)), dusts were superior to sprays for the control of aphids, but sprays were better for the control of mites. There was no difference between low- and high-gallonage sprays against either pest. With low-gallonage sprays, control of both aphids and mites increased as the amount of active ingredient per acre was increased; but with higher strengths, the improvement did not justify the additional expense. In applying more toxicant per acre, there was no significant difference between increasing the gallonage per acre, and increasing the strength of the spray.

Experiments to determine the comparative effectiveness of dusts and low- and high-gallonage sprays were conducted on strawberries in southern California from 1954 through 1957. The insecticides were applied at several dosages for the control of the strawberry aphid (*Pentatrichopus fragaefolii* (Ckll.)) and the two-spotted spider mite (*Tetranychus telarius* (L.)).

Plots were 30 feet long and one bed wide. The beds were either $2\frac{1}{3}$ feet or $3\frac{1}{3}$ feet wide. The plots were arranged in randomized blocks with at least four replicates. Dusts were applied with a rotary hand duster, low-gallonage sprays with a high-pressure mist sprayer, and high-gallonage sprays with a knapsack sprayer. Aphid tests were conducted from February to April, and mite tests from February to July. Counts of aphids or mites surviving treatments were made on the underside of five leaflets per plot at weekly intervals, when possible. Treatments usually were repeated when the number averaged five or more per leaflet. In most tests the average number of mites for all replicates of one material determined whether the application was to be repeated and all replicates were retreated, but in Experiment

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2-1956 only the individual plots were retreated when they exceeded five mites per leaflet.

The following insecticides were used in these experiments:

Aramite® (2-(*p-tert*-butylphenoxy)-1-methylethyl 2-chloroethyl sulfite) Emulsion concentrate (EC) 2 and 4.8 pounds per gallon, dust 3%
Delnav® (2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate) 68%–70% and related materials) EC 2 and 4 pounds per gallon, dust 3%
Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) EC 2 pounds per gallon, dust 4%
Dow ET-15 (*O*-methyl *O*-2,4,5-trichlorophenyl phosphoramidothioate) EC 2 pounds per gallon, dust 5%
Kelthane® (4,4′-dichloro-*alpha*-(trichloromethyl)-benzhydrol) EC 1.6 pounds per gallon, dust 3.7% and 5%
Lindane (EC 1.6 pounds per gallon, dust 1%)
Malathion (EC 5 pounds per gallon, dust 4%)
Nicotine (Nicotine sulphate 40% (4 pounds per gallon), dust 4% nicotine alkaloid)
Sulphenone (*p*-chlorophenyl phenyl sulfone) EC 4 pounds per gallon
Ovex (EC 2 pounds per gallon, dust 10%)
Ronnel (EC 2 pounds per gallon, dust 5%)
TEPP (EC 4 pounds per gallon, dust 1%)
Trithion (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate) EC 4 pounds per gallon, dust 2%

STRAWBERRY APHID.—*1954 Experiment.*—The insecticides tested were Diazinon, TEPP, and nicotine. Applications were made on March 15 and April 9 and counts on March 31 and April 13 and 31. There were four replicates, and each insecticide was applied at three dosages and by three methods; as dusts, and as low- and high-gallonage sprays. The high-gallonage sprays of TEPP and nicotine contained 8 ounces of Triton B-1956 spreader per 100 gallons. The results from the individual treatments together with the strengths and dosages of the materials used are shown in table 1. The per cent reduction in aphids for the different classes of treatments (36 replicates each) is shown below.

	Per Cent
Insecticides (averages for all dosages and methods):	
Diazinon	96
TEPP	86
Nicotine	54
Dosages (averages for all insecticides and methods):	
High	86
Medium	82
Low	70
Methods of application (averages for all insecticides and dosages):	
Dusts	86
Low-gallonage sprays	76
High-gallonage sprays	74
L.S.D. at 5% level	9

Diazinon was superior to TEPP, and TEPP to nicotine. Dusts were superior to sprays, but there was no significant difference between low- and high-gallonage sprays. High and medium dosages were similar in effectiveness and were superior to low dosages.

1955 Experiment.—Lindane, TEPP, and nicotine were tested in 1955. Applications were made on March 2 and 22, and there were six replicates. Aphid counts were made on March 9, 16 and 30. The results of the individual treatments are shown in table 1. The per cent reduction in aphids for the different classes of treatments (54 replicates) is shown below.

	Per Cent
Insecticides (averages for all dosages and methods):	
Lindane	94
TEPP	88
Nicotine	71
Dosages (averages for all insecticides and methods):	
High	93
Medium	88
Low	78
Methods of application (averages for all insecticides and dosages):	
Dusts	89
Low-gallonage sprays	88
High-gallonage sprays	78
L.S.D. at 5% level	4

Lindane was superior to TEPP, and TEPP to nicotine. Dusts and low-gallonage sprays were superior to high-gallonage sprays. High dosages were better than medium, and medium better than low.

1956 Experiment.—This experiment was designed as a full-season test for the control of the strawberry aphid and the two-spotted spider mite. Diazinon, Delnav, TEPP, and Trithion were applied. (See discussion of 1956 test results on the two-spotted spider mite later in this report.) Aphid results are based on the two applications, on February 22 and April 4, and on the counts made February 28, March 7, 15, 21, and 28, and April 19 and 24. The results for the individual treatments are shown in table 1. The per cent reduction in aphids for the different classes of treatments (36 replicates for materials, and 48 replicates for methods of application and rates per acre) is shown below.

	Per Cent
Insecticides (averages for all dosages and methods):	
Diazinon	95
Trithion	94
Delnav	85
TEPP	75
L.S.D. at 5% level	5
Dosages (averages for all insecticides and methods):	
High	86
Medium	82
Low	70
Methods of application (averages for all insecticides and dosages):	
High-gallonage sprays	90
Dusts	87
Low-gallonage sprays	84
L.S.D. at 5% level	4

Diazinon and Trithion each were superior to Delnav, and Delnav to TEPP. High-gallonage sprays were better than low-gallonage sprays, but dusts were intermediate and similar in effectiveness. High and medium dosages were similar to each other in effectiveness for aphid control, and each was superior to low dosages.

1957 Experiment.—In this experiment, low-gallonage sprays were compared with high-gallonage sprays. Also, using the same insecticide concentration, the gallonage per acre was increased two and three times by applying more spray (water). For comparison, the amount of insecticide per acre was increased two, three, and four times

Table 1.—Control of the strawberry aphid with insecticides applied at different strengths and rates as dusts and as low- and high-gallonage sprays, 1954–1957.

Insecticide	Rate per Acre per Application: Gallons of Spray	Pounds of Dust	Pounds of Toxicant	Number of Surviving Aphids per Leaflet
	1954 Experiment			
Diazinon	—	34	1.3	0.1
	—	22	0.9	.5
	—	11	.4	1.3
	18	—	1.4	0.5
	12	—	1.0	.3
	6	—	0.5	.8
	169	—	1.4	.2
	113	—	0.9	.4
	56	—	.4	2.4
TEPP	—	74	.7	0.2
	—	49	.5	.8
	—	25	.2	2.8
	17	—	.7	2.7
	11	—	.4	3.7
	6	—	.2	4.1
	156	—	.6	2.7
	104	—	.4	3.0
	52	—	.2	4.8
Nicotine alkaloid	—	59	2.4	3.0
	—	39	1.6	6.1
	—	20	0.8	10.4
Nicotine sulfate	16	—	2.6	6.8
	11	—	1.7	8.6
	5	—	0.9	15.7
	148	—	2.2	10.1
	99	—	1.5	10.4
	49	—	0.7	13.2
L.S.D. at 5% level	—	—	—	5.3
Check	—	—	—	20.4
	1955 Experiment			
Lindane	—	58	0.6	0.2
	—	39	.4	.9
	—	20	.2	1.6
	16	—	.6	0.1
	11	—	.4	.1
	5	—	.2	.4
	145	—	.6	.2
	102	—	.4	.2
	47	—	.2	1.3
TEPP	—	61	.6	0.5
	—	40	.4	.9
	—	20	.2	2.3
	18	—	.2	0.6
	12	—	.5	1.1
	6	—	.2	1.5
	163	—	.6	2.0
	106	—	.4	2.2
	53	—	.2	3.5
Nicotine alkaloid	—	61	2.4	1.4
	—	40	1.6	2.3
	—	20	0.8	3.2
Nicotine sulfate	17	—	2.7	1.7
	12	—	1.9	2.9
	6	—	1.0	5.4
	155	—	2.5	2.1
	99	—	1.6	8.2
	52	—	0.8	7.2
L.S.D. at 5% level	—	—	—	1.6
Check	—	—	—	13.4
	1956 Experiment			
Diazinon	—	31	1.2	1.0
	—	23	0.9	3.0
	—	11	.4	3.0
	18	—	1.5	0.3
	12	—	1.0	.2
	6	—	0.5	.5

Table 1.—(*Continued*)

Insecticide	Rate per Acre per Application: Gallons of Spray	Pounds of Dust	Pounds of Toxicant	Number of Surviving Aphids per Leaflet
	147	—	1.2	.2
	101	—	0.8	.2
	51	—	.4	.3
Trithion	—	45	0.9	1.1
	—	27	.5	1.6
	—	13	.3	2.1
	15	—	.8	0.4
	12	—	.6	1.1
	6	—	.3	2.0
	154	—	.8	0.8
	102	—	.5	.8
	51	—	.3	1.5
Delnav	—	49	1.5	1.4
	—	33	1.0	2.2
	—	16	0.5	5.8
	16	—	1.6	1.5
	12	—	1.2	5.2
	7	—	0.7	5.5
	151	—	1.3	1.3
	101	—	1.0	1.0
	50	—	0.5	2.9
TEPP	—	65	.6	1.9
	—	43	.4	4.0
	—	21	.2	3.8
	18	—	.7	7.0
	12	—	.5	4.1
	6	—	.2	9.2
	150	—	.6	3.4
	100	—	.4	4.2
	50	—	.1	7.6
L.S.D. at 5% level	—	—	—	2.9
Check	—	—	—	19.8
	1957 Experiment			
Diazinon	31	—	0.4	0.6
	20	—	.2	1.0
	10	—	.1	2.6
	11	—	.5	0.6
	11	—	.4	.6
	11	—	.3	1.4
	154	—	.4	0.6
	103	—	.3	.8
	53	—	.1	2.1
	51	—	.5	0.3
	48	—	.4	.8
	49	—	.2	1.2
L.S.D. at 5% level	—	—	—	0.7

by using more insecticide in the minimum amount for both low-gallonage (10 gallons) and high-gallonage (50 gallons) sprays.

Diazinon emulsifiable concentrate (2 pounds per gallon) was used at about 2, 4, 6, and 8 ounces of toxicant per acre on April 10. There were eight replicates, and counts were made on April 15 and 25 and May 1. The results of the individual treatments are shown in table 1. Aphid control was satisfactory with all treatments, as the population averaged less than five per leaflet. No check plots were included, but it is common for untreated plots to average 20 or more aphids per leaflet at this time of year. The numbers of aphids per leaflet for the different classes of treatments are shown below.

Spray gallonage (average for all dosages):	
High	1.0
Low	1.1
L.S.D. at 5% level	0.3

Dosage increase (average of two methods of applying more toxicant per acre):	
By applying more spray per acre	0.8
By applying more toxicant in a minimum amount of spray per acre	1.0
L.S.D. at 5% level	0.3
Dosages (average of both gallonages):	
8 ounces per acre	0.4
6 ounces per acre	.7
4 ounces per acre	1.3
2 ounces per acre	2.4
L.S.D. at 5% level	0.5

High-gallonage sprays were better than low-gallonage sprays, but the difference was not significant. Somewhat better control was obtained when the dosage was increased by applying more spray per acre than by adding more toxicant and applying constant minimum amounts of spray per acre, but this difference was not significant. Control was increased with each increase in dosage, but the increase from 6 to 8 ounces was not significant.

TWO-SPOTTED SPIDER MITE.—*1954 Experiment.*—Aramite and ovex dusts and low-gallonage sprays were tested. The initial application was made to all plots on April 27, and subsequent applications were made to those plots that averaged five or more mites per leaflet. Counts were made weekly during May and June and at 2-week intervals during July. For the dusts four applications were required at the 30-pound and three at the 60-pound rate. For the sprays, three applications were required at the 10-gallon and two at the 20-gallon rate. There were four replicates. The results of the individual treatments are shown in table 2. The numbers of mites per leaflet for the different classes of treatments are shown below.

Insecticides (averages for both dosages and methods):	
Ovex	4.5
Aramite	5.4
Dosage (averages for both insecticides and methods):	
Heavy	3.6
Light	6.3
Methods of application (averages for both insecticides and dosages):	
Sprays	3.4
Dusts	6.5
L.S.D. at 5% level	2.3

Low-gallonage sprays were superior to dusts, and required less toxicant per acre and fewer applications. Ovex was better than Aramite, but the difference was not significant, and the much greater amount of Ovex used might be a detriment from a residue standpoint. Heavy dosages were better than light ones; they required more toxicant per acre, but fewer applications. Growers would usually rather pay more for material, make fewer applications, and get better control by using heavy dosages, but from a residue standpoint light applications might be preferred.

1955—Experiment 1.—Aramite, ovex, and Kelthane were each used as dusts and as low-gallonage sprays, and Delnav was used as a low-gallonage spray. There were six replicates, and the first application was made on March 31. Weekly counts were made thereafter until July 12, and any treatment that averaged five or more mites per leaflet was repeated so that as many as eight applications were made during this 14-week period. The results of the individual treatments are shown in table 2. The numbers of mites per leaflet for the different classes of treatments are shown below.

Insecticides (averages for all dosages and methods):	
Kelthane	2.6
Aramite	5.4
Ovex	6.0
L.S.D. at 5% level	1.9
Sprays only:	
Kelthane	1.9
Ovex	2.6
Aramite	3.0
Delnav	5.7
L.S.D. at 5% level	2.7
Dosages (averages for all insecticides (except Delnav) and methods):	
High	3.8
Low	5.9
L.S.D. at 5% level	1.4
Methods of application (averages for all insecticides (except Delnav) and dosages):	
Sprays	2.5
Dusts	6.8
L.S.D. at 5% level	1.6

When both dusts and sprays were averaged, Kelthane was superior to Aramite and ovex, and required fewer applications but more toxicant per acre. When sprays only were compared, there was no difference between Kelthane, ovex, and Aramite, and all three were superior to Delnav, but less Delnav was used per acre. Sprays were superior to dusts, and required fewer applications and less toxicant per acre. High dosages were better than low dosages and required fewer applications per acre but more toxicant for the season.

1955—Experiment 2.—Malathion, TEPP, ronnel, and Dow ET-15 were applied as dusts and low-gallonage sprays. One application was made on May 4, and four counts were made at weekly intervals thereafter. There were four replicates. The results of the individual treatments are shown in table 2. The numbers of mites per leaflet for the different classes of treatments are shown below.

Insecticides (averages for all dosages and methods):	
Dow ET-15	0.6
Malathion	5.5
TEPP	6.5
Ronnel	8.8
L.S.D. at 5% level	2.8
Dosages (averages for all dosages and methods):	
Low	4.6
High	6.0
L.S.D. at 5% level	2.0
Methods of application (averages for all insecticides and dosages):	
Sprays	4.5
Dusts	6.2
L.S.D. at 5% level	2.0

Dow ET-15 was the only material that kept the mites down to not more than five per leaflet for nearly a month, and it was significantly better than the other materials. Malathion was better than ronnel. There was no significant difference between dusts and sprays or between the low and high dosages.

1956—Experiment 1.—Data on rates per acre and the replications are shown in table 1. Mite counts began on

Table 2.—Control of the two-spotted spider mite on strawberry with insecticides applied at different strengths and rates of dusts or sprays, 1954–57.

Insecticide	Rate per Acre: Gallons of Spray	Rate per Acre: Pounds of Dust	Rate per Acre: Pounds of Toxicant	Number of Applications for the Season	Number of Surviving Mites per Leaflet
	1954 Experiment				
Ovex	20	—	6.1	2	1.2
	10	—	3.0	3	2.7
	—	60	6.0	3	4.9
	—	30	3.0	4	7.2
Aramite	19	—	1.8	2	3.3
	9	—	0.9	3	6.4
	—	60	1.8	3	5.0
	—	30	0.9	4	6.8
L.S.D. at 5% level	—	—	—	—	4.5
	1955—Experiment 1				
Kelthane	20	—	2.2	2	2.5
	10	—	1.1	4	1.2
	—	38	1.9	4	3.0
	—	19	1.0	5	3.6
Ovex	20	—	4.1	2	2.2
	10	—	1.9	3	3.0
	—	42	4.2	6	8.2
	—	20	2.0	8	10.8
Aramite	20	—	1.2	3	2.5
	10	—	0.6	5	3.5
	—	40	1.2	5	5.3
	—	21	0.6	7	10.2
Delnav	21	—	.8	3	4.0
	10	—	.4	4	7.4
L.S.D. at 5% level	—	—	—	—	3.8
	1955—Experiment 2				
Dow ET-15	22	—	2.2	1	0.2
	11	—	1.1	1	0.8
	—	36	1.8	1	.4
	—	18	0.9	1	1.1
Ronnel	21	—	2.1	1	7.6
	10	—	1.0	1	4.2
	—	36	1.8	1	14.4
	—	18	0.9	1	9.1
TEPP	18	—	.7	1	8.5
	9	—	.4	1	4.7
	—	36	.4	1	5.8
	—	18	.2	1	6.9
Malathion	22	—	2.2	1	5.4
	11	—	1.1	1	5.0
	—	56	2.2	1	6.2
	—	28	1.1	1	5.4
L.S.D. at 5% level	—	—	—	—	5.6
	1956 Experiment				
Kelthane	10	—	3.3[a]	2	1.4
	9	—	2.3	2	1.8
	10	—	1.8	2	2.1
	—	40	3.9	3	3.4
	—	29	3.5	3	4.8
	—	20	2.5	3	4.5
Aramite	10	—	4.4	3	3.4
	10	—	3.4	4	4.7
	10	—	2.8	5	6.8
	—	40	4.4	3	3.5
	—	30	3.1	3	4.9
	—	21	2.7	4	7.5
Sulphenone	9	—	6.2	4	4.0
	18	—	8.3	3	4.9
	10	—	5.2	5	6.5
L.S.D. at 5% level	—	—	0.9	1	2.5
	1957 Experiment				
Kelthane	32	—	1.3	1	0.8
	22	—	0.9	1	3.9
	11	—	.4	1	8.8
	10	—	1.7	1	0.7
Kelthane	10	—	1.2	1	1.6
	10	—	0.8	1	3.9
	146	—	1.2	1	2.2
	98	—	0.8	1	3.2
	50	—	.4	1	10.5
	48	—	1.6	1	2.5
	48	—	1.2	1	2.2
	50	—	0.8	1	7.2
L.S.D. at 5% level	—	—	—	—	4.6

[a] This column gives total for all treatments in 1956 experiment.

April 19, and 12 counts were made up to July 25. During this period the insecticide applications were repeated on all plots for each insecticide whenever the population exceeded five per leaflet. The numbers of mites per leaflet for the different classes of treatments are given below.

Insecticides (averages for all methods and dosages):	
Trithion	2.5
Delnav	4.3
Diazinon	7.0
TEPP	10.1
L.S.D. at 5% level	1.6
Dosages (averages for all methods and insecticides):	
Medium	4.8
High	5.1
Low	7.9
L.S.D. at 5% level	1.4
Methods of application (averages for all insecticides and dosages):	
High-gallonage sprays	5.4
Low-gallonage sprays	5.4
Dusts	7.1
L.S.D. at 5% level	1.4

Trithion was the most effective and required the fewest applications for the season. Delnav was better than Diazinon, and Diazinon better than TEPP. High- and low-gallonage sprays were about equal and were better than dusts and required fewer applications. Medium and high dosages were about equal and were superior to and required fewer applications than low dosages.

1956—Experiment 2.—Kelthane, Aramite, and Sulphenone were used at three strengths as low-gallonage sprays, and Kelthane and Aramite also as dusts. There were six replicates, and the initial application was made on May 2. Ten weekly counts were made thereafter until July 26. Individual plots were retreated on the day following the counts if they averaged five or more mites per leaflet. In previous tests retreatment was on a material basis and all replicates were retreated when there were five or more mites per leaflet.

Severe burn resulted from the initial application of the high strength of Sulphenone so subsequent applications were made at half the initial strength. The results of the individual treatments are shown in table 2. The numbers of mites per leaflet for the different classes of treatments are shown in the following list.

Insecticides (average of all dosages):	
Kelthane sprays	1.8
Kelthane dusts	4.2
Aramite sprays	5.0
Sulphenone sprays	5.2
Aramite dusts	5.3
L.S.D. at 5% level	1.5
Dosages (averages of all insecticides (except Sulphenone) and methods):	
High	3.3
Medium	4.0
Low	5.5
L.S.D. at 5% level	1.3
Methods of applications (averages of all insecticides (except Sulphenone) and dosages):	
Sprays	3.4
Dusts	4.8
L.S.D. at 5% level	1.0

Kelthane sprays were the most effective treatment, and required less toxicant per acre and fewer applications, all of which were significant. In the analysis of Kelthane and Aramite, sprays were better than dusts and required fewer applications and less toxicant, but the latter difference was not significant. The high and medium dosages gave better control than the low dosage; the high dosage required more toxicant but fewer applications.

1957 Experiment.—This experiment on mite control was similar to the 1957 experiment on aphid control. Kelthane was applied as low- and high-gallonage spray on May 2 and counts were made on May 6, 13, and 22. There were eight replicates. Dosages were increased by applying more spray per acre, or by keeping the amount of spray per acre at a minimum level and increasing the strength. Results of the individual treatments are shown in table 2.

Shown below are several combinations of the data from the various replicates.

Spray gallonage (average of all dosages):	
Low	3.3
High	4.6
L.S.D. at 5% level	1.9
Dosage increase (averages of 2 methods of applying more toxicant per acre):	
By applying more spray per acre	2.5
By applying more toxicant in a minimum amount of spray per acre	3.7
L.S.D. at 5% level	2.3
Dosages (average for both gallonages):	
1.6 pounds per acre	1.6
1.2 pounds per acre	1.9
0.8 pound per acre	5.5
.4 pound per acre	9.7
L.S.D. at 5% level	3.3

Low-gallonage sprays were better than high-gallonage sprays, but the difference was not significant. Better control was obtained when the amount of toxicant was increased by applying more spray per acre than when more toxicant was used in the same amount of spray, but the difference was not significant. Mite control was improved by each increase in dosage, but the improvement between 1.2 and 1.6 pounds per acre was not significant.

DISCUSSION.—As both the strawberry aphid and the two-spotted spider mite are found on the underside of the leaves of strawberry plants, frequently at the same time, it would be logical to assume that a method of application superior for the control of one would be superior for the control of the other. However, in these experiments dusts were more effective than sprays against aphids and sprays were the more effective against mites.

The superiority of dusts for aphid control was noted in 1954 when the results for the three insecticides were averaged. However, for the individual materials, the difference was only apparent with nicotine, although it approached significance with TEPP, and there was little or no difference between dusts and sprays of Diazinon. In 1955 dusts and concentrate sprays were equally effective when the three materials were averaged, but nicotine and TEPP dusts were better than sprays, whereas lindane sprays were as effective as dusts. In 1956 when Diazinon, Trithion, Delnav, and TEPP were used, there was no difference between dusts and sprays. Since dusts were generally better than sprays, the difference might have been due to the nature of the insecticide rather than the method of application. Insecticides that depend on a fuming action for control appeared to be more effective as dusts.

The difference between low- and high-gallonage sprays for aphid control varied with the year. In 1954 and 1957 there was no difference, in 1955 low-gallonage sprays were better, and in 1956 high-gallonage sprays were superior. In 1957 an increase of toxicant per acre by applying more spray was no better than by increasing the strength. However, the highest dosages were not always justified by the improvement in control obtained.

In mite control sprays were better than dusts, required fewer applications for the season, and less toxicant per acre. Some compounds were less phytotoxic in dusts than in sprays, but were less desirable because they left a visible residue on the fruit and obscured the natural gloss of the berries. As there was no consistent difference between low- and high-gallonage sprays, low-gallonage sprays would be preferred because of their ease and speed of application. High and medium strengths gave better control than low strengths and required fewer applications per season, but used more toxicant per acre. As there was no appreciable difference in control between high and medium strengths, the latter would probably be preferred, since they use less toxicant, but would require more applications for the season. However, a few growers prefer to make heavy applications even though the extra cost of the insecticide is not balanced by the fewer applications.

Mite control increased with the amount of toxicant per acre. In applying more toxicant per acre, there was no significant difference between increasing gallonage per acre and the strength of the spray.

Phytotoxicity resulted from the use of emulsion sprays of Sulphenone, Aramite, TEPP, and ovex, but none was evident from the use of Kelthane, Trithion, Delnav, and Diazinon.

That the best dust formulations were not perfected for some of these materials at the time they were used in these tests was evidenced by the fact that Diazinon and Delnav dusts have since been withdrawn by the manufacturers. However, better dusts would only tend to emphasize that dusts are better than sprays for aphid control, and as the sprays were outstandingly better than dusts for mite control, it seems unlikely that better dust formulations would make much difference in the final results.

An Evaluation of Certain Compounds as Feeding Deterrents Against the Smaller European Elm Bark Beetle, *Scolytus multistriatus*[1]

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Abstract

The smaller European elm bark beetle, *Scolytus multistriatus*, was reared in elm logs in culture chambers fashioned from 55-gallon lever-lock drums. Feeding cages were designed and 42 compounds were tested as feeding deterrents against the beetle. None were as effective in reducing feeding at concentrations of 20,000 p.p.m. as was DDT. Four chemicals (decanoic acid, N,N-diethyl-m-toluamide, 3,4-diethoxybenzaldehyde and dimethyl phthalate) proved to be very effective in combination with DDT. Concentrations of 10,000 p.p.m. of the deterrent together with a similar amount of DDT were in each case equal or superior to 20,000 p.p.m. DDT in effectiveness. The same compounds at 5,000 p.p.m., in combination with DDT at 5,000 p.p.m. each, were also equal to 20,000 p.p.m. DDT in reducing feeding.

The most effective method of controlling the spread of Dutch elm disease is to suppress the population of the smaller European elm bark beetle, *Scolytus multistriatus* (Marsh.). This may be accomplished by removing and burning all infested trees and logs and all elms in a weakened condition. Such measures are costly and, therefore, are not practiced on a large scale. Small areas, even if freed of the fungus and the beetle, are in danger of reinfestation and subsequent reinfection from adjacent areas where the disease and the beetle still exist. Insecticides have been employed to help prevent infestations. DDT has proved to be most effective (Whitten & Swingle 1958), although dieldrin, lindane, methoxychlor (Matthysse *et al.* 1954), and a systemic insecticide, Chipman R-6199 (Al-Azawi & Casida 1958) have shown some degree of promise. Work by Nielsen (1959) has suggested the combination of Arochlor and BHC or lindane for control of this beetle.

The concentration of DDT used to kill the beetle is high, between 1% and 2%. Outbreaks of scale insects, aphids and mites often follow the use of DDT. This generally is the result of a reduction of their parasites or predators by the insecticide. Reports of bird toxicity frequently follow the use of this standard bark beetle spray (Barker 1958, DeWitt 1958, Wallace 1959).

Because of these and other problems inherent with the use of DDT, it was felt advisable to explore other areas of control. One such area is in the field of repellents, or feeding deterrents as defined by Dethier *et al.* (1960). A program of evaluating compounds as feeding deterrents for the smaller European elm bark beetle was therefore initiated.

A survey of the literature reveals very little information on repellents or deterrents for *Scolytus* beetles. Snapp (1941) reported that the shot-hole borer (*Scolytus rugulosus* (Ratz.)) was repelled from peach by a thick coat of whitewash. Struble (1937) observed that the fir engraver beetle (*Scolytus ventralis* Lec.) was repelled by balsam. Whitten (1942) made a rather extensive study of materials as repellents for the two elm bark beetles, *Scolytus multistriatus* and *Hylurgopinus rufipes* (Eichh.). Orthodichlorobenzene, naphthalene, monochloronaphthalene and pentachlorophenol all effectively prevented infestation of elm logs by these two beetles, but these were not tested as repellents on live trees.

Culture of the Beetles.—Data from these studies confirmed that it is possible to maintain a near-continuous supply of the smaller European elm bark beetles for test purposes. A colony of this beetle was established and maintained in eight culture chambers fashioned from 55-gallon lever-lock drums after a plan provided by Dr. William B. Becker (fig. 1). Three 8-inch holes were cut in the side of each drum and a funnel made of 30-mesh screen was soldered over each opening. A bottle was placed over the opening of each funnel. Strips of paper were added to provide footing for the beetles, thus preventing them from slipping to the lowest points of the jars.

The only report of a culture chamber of this design appearing in the literature is by Griswold (1948). The main distinction between the two types of chambers is that those described by Griswold were made from 5-gallon paint buckets or large trash cans, while the ones designed by Becker were made from 55-gallon drums.

Noninfested elm logs, about 30 inches long and from 5 to 18 inches in diameter, were placed in each chamber as it was ready to be stocked with beetles. Two beetles per 3 square inches of bark surface were arbitrarily used in stocking the chambers.

The chambers were housed in the basement of the University greenhouse where the temperature was maintained at an average of 75° ± 9° F. The humidity within the chambers was maintained at about 74%. This was accomplished by opening the chambers to lower the humidity and by adding wet paper towels to increase it. When the relative humidity rose above 80%, a fungus growth on the logs became a problem; when it fell below 56%, adult emergence was retarded.

Cage-Test Procedure.—Six wood-frame cages (fig. 2) were built to study the tactile and gustatory responses of the beetles to the chemicals being evaluated. No attempt was made to distinguish between these two responses. The cages were 15½ inches long, 10¼ inches high and 9¼ inches wide outside dimension, and 13 inches long, 10 inches high, and 8 inches wide inside dimension. The top and ends were covered with a fine mesh screen. The front and back panels contained glass to permit better observation. The base was elevated 2 inches and supported 12

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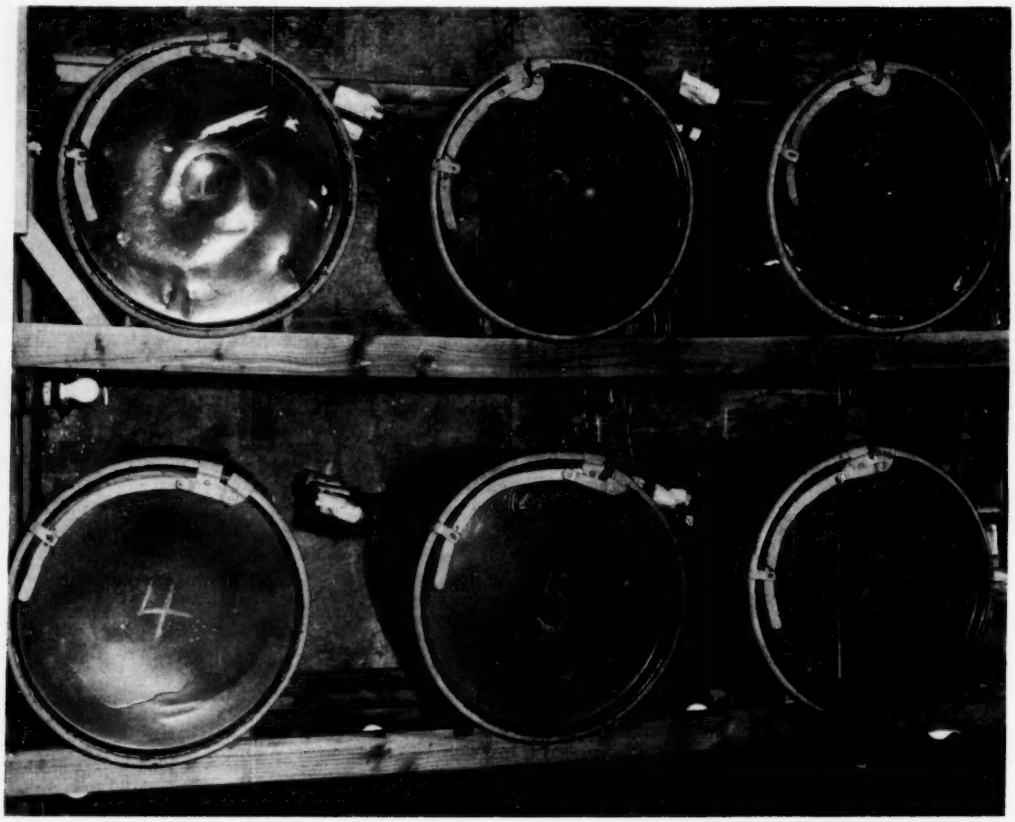

FIG. 1.—Chambers used for rearing bark beetles.

vials fitted with corks so arranged as to hold a twig securely. Removable bases permitted easy access to the vials. Five cages were used for the study of each chemical or concentration of chemical and a sixth was used as a control. Two twigs of American elm (*Ulmus americana* L.) were dipped in an acetone solution of the candidate chemical, and were placed randomly in two of the 12 vials in each cage. Five chemicals were evaluated in each test. Two twigs in each test cage were dipped in water only (preliminary tests indicated acetone had no effect on feeding). Twelve twigs were dipped in water and placed in the sixth (control) cage. Each position was numbered to make readings easier.

Fifty beetles were introduced into each cage. The cages were draped with two thicknesses of heavy black muslin, to prevent the beetles from responding to light, and were placed on a bench containing moist sand in a section of the greenhouse where the temperature ranged from 72° to 98° F. It was necessary to maintain a relatively high humidity (above 50%) in order to prevent high beetle mortality. The number of beetles on each twig and the amount of feeding thereon was observed at $\frac{1}{3}$, 2, 8 and 24 hours. To facilitate record-taking, the following arbitrary index of feeding was established: If the volume of bark eaten was approximately equal to the volume of the

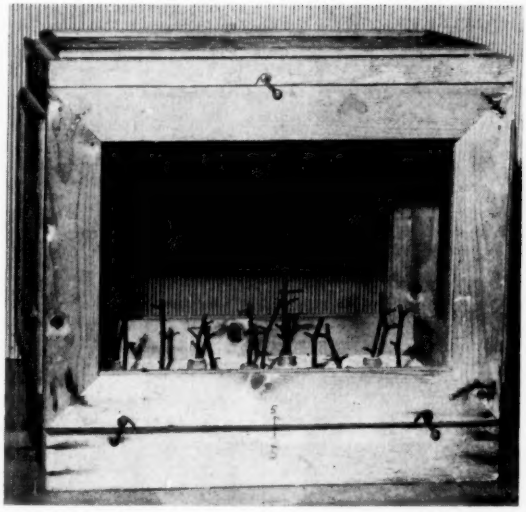

FIG. 2.—Cage for studying response of bark beetles to candidate chemical deterrents.

Table 1.—Cage tests of candidate feeding deterrents against *Scolytus multistriatus*—24-hour results.

CHEMICAL	PER CENT REDUCTION IN FEEDING OVER CONTROL AT P.P.M. SHOWN		
	250,000	20,000	10,000
AC-5812[a]		0	
AC-8331		0	
AC-8839		0	
AC-13350		14	
AC-15351		0	
AC-15578		53*	
AC-15891		31	
AC-26063		0	
AC-29756		55*	
AC-CL24055		0	
Aluminum potassium sulfate		−26	
Benzyl benzoate	88*		
N-n-Butylacetanilide		59*	
Camphene	−54		
Camphor	−22		
Carbolic acid	67*		
Cedar leaf oil	0		
Cinnamyl alcohol		46*	
Crag Fly Repellent #2060		19*	
Creosote	75*		
Cresol	96*		
DDT	90*	90*	79*
Decanoic acid		78*	61*
Dieldrin	74*		
3,4-Diethoxybenzaldehyde	97*	41*	60*
N,N-Diethyl-m-Toluamide	100*	58*	28
N,N-Diethyl-p-Toluamide	65*		
Dimethyl phthalate		82*	68*
Di-n-butyl succinate		50*	
Ethylhexanediol		13	34
Furfural	−25		
Indalone	97*	62*	38
Lime, Spray	75*		
Methyl salicylate	68*		
Naphthalene	73*		
Nicotine sulfate	65*		
M-Nitrobenzaldehyde	93*		
Paradichlorobenzene	10		
Phenothiazine	80*		
Pine Oil	31		
Pyridine	2		
Tetramethyl thiuram disulfide	69*		

[a] AC—American Cyanamid Company numbered compounds.
* Significance at the 0.05 level indicated.

beetle, the index value was 1; thus, a feeding index of 2 would indicate a volume fed upon equal to twice the size of an average beetle.

STATISTICAL ANALYSIS.—In the analysis of the results of the cage tests, the mean feeding index for a given chemical was recorded at ½, 2, 8 and 24 hours. The standard deviation of the mean and the standard error of the mean were calculated for the chemical and it was then compared with the average of the control for significance at the 0.05 level of probability. In this paper only the results of the 24-hour observations are presented because they represent the greatest effectiveness of the chemicals. Inasmuch as there was no way to compare chemicals from different tests in a statistically approved manner, any chemical to be compared with another had to be included in the same test.

RESULTS.—Forty-two chemicals were evaluated, 22 of which showed some degree of feeding inhibition (table 1). Twelve of these compounds showed significant feeding inhibition at concentrations of 20,000 p.p.m. or 2% (the concentration of DDT in the standard bark beetle spray). Five of these showed promise. They were decanoic acid, dimethyl phthalate, meta delphene, indalone and 3,4-diethoxybenzaldehyde (table 2).

Table 2.—Cage tests of feeding deterrents in combination with DDT against *Scolytus multistriatus*—24-hour results.

CHEMICAL	CONCENTRATION (P.P.M.)	PER CENT REDUCTION IN FEEDING OVER CONTROL AT INDICATED CONCENTRATION OF DDT (P.P.M.)				
		0	2,500	5,000	10,000	20,000
Decanoic acid	2,500			85		90
	5,000		18*	88	95	
	10,000			95	97	
	20,000	78				
3,4-Diethoxybenzaldehyde	2,500			87		
	5,000		76	89	93	
	10,000			93	94	
	20,000	41				
N,N-Diethyl-m-Toluamide	2,500			79		
	5,000		89	91	92	
	10,000			91	95	
	20,000	58				
Dimethyl phthalate	2,500			87		
	5,000		76	91	95	
	10,000			92	96	
	20,000	82				
Indalone	5,000			70	92	
	10,000			80	91	
	20,000	62				

* No significance at the 0.05 level indicated.

None of the above mentioned chemicals were as effective in reducing feeding as were either 20,000 or 10,000 p.p.m. of DDT. It was observed with DDT, however, that the beetles began to feed soon after being introduced into the cages. They would consume a toxic dose and die. On the other hand, with the twigs treated with deterrents, the beetles would wander about for most of the test period, then a few beetles would start to feed. It was decided to combine the two to see if there would be a complementary action.

It was found that decanoic acid at 10,000 p.p.m. together with an equal amount of DDT was significantly better in reducing feeding at the 0.05 level than either 20,000 or 10,000 p.p.m. of DDT alone. A test using lower concentrations of decanoic acid and DDT in combination showed that a combination of 5,000 p.p.m. of each was equal to 20,000 p.p.m. of DDT alone in reducing feeding.

A combination of dimethyl phthalate and DDT at 10,000 p.p.m. of each was equal to 20,000 p.p.m. of DDT in effectiveness and was superior to 10,000 p.p.m. of DDT. Indalone was similar to dimethyl phthalate in this respect.

The combination of meta delphene and DDT at 10,000 p.p.m. of each, consistently reduced feeding more than either 1% or 2% DDT alone; however, this difference was never significant.

The results with 3,4-diethoxybenzaldehyde in combination with DDT were variable. At times the combination seemed effective in reducing feeding, but at no time was the combination superior to 2% DDT alone.

A comparison of four of these combinations of DDT plus a deterrent at 10,000 p.p.m. of each material showed that decanoic acid and DDT gave the greatest reduction

in feeding. This mixture was not significantly better than the other combinations however. The combinations tested were: dimethyl phthalate-DDT; meta delphene-DDT; and 3,4-diethoxybenzaldehyde-DDT, all at 10,000 p.p.m. of each.

DISCUSSION.—Cage tests in the laboratory indicated that combinations of a feeding deterrent with DDT are advantageous. It is possible that these advantages will not hold true in the field. Observations of the phytotoxicity and weathering properties of these combinations will have to be made. It will also be necessary to observe side effects such as the effects of the chemicals on aphid and mite populations and toxicity to birds. The feeding deterrents might make a better showing in the field than they did in the laboratory because of the fact that the beetles were trapped in the vicinity of the chemical in the feeding cages. In nature the beetles could depart from the treated area and not return. In any event, the next logical step is the evaluation of these feeding deterrents in the field, both alone and in combination with DDT, on a small scale test plot. Such a program is already underway.

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Emergence of Phorate-Treated Cotton Seed as Affected by Substrate Moisture and Temperature[1]

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ABSTRACT

Deltapine-15 cotton seed with and without phorate were planted in washed river sand at two moisture levels and four temperatures. Regardless of treatment, seedling emergence was favored by a moisture level of 50% of field capacity as compared with one of 75%. The final stand counts at 86° and 77° F. were comparable for the phorate-treated and untreated seed. Phorate, however, caused a marked reduction in the rate and total emergence at both 72° and 67°.

A similar experiment with another lot of seed of the same variety showed that seedling emergence with and without phorate seed treatment were comparable at 80° and 67°.

It was concluded that reduction in emergence in phorate-treated seed often found may be due to inferior quality of seed as well as to low temperatures and high levels of soil moisture.

Phorate applied to the seed or soil for the control of insects on cotton has a number of advantages over insecticides applied as sprays and dusts. Some of these are: few beneficial insects are killed; less hazard to the applicator; ease of application; and less frequent applications of the toxicant are required. However, field (Adkisson 1958, Parencia *et al.* 1957, and Reynolds *et al.* 1957) and laboratory (Lindquist *et al.* 1960) data have shown that phorate applied to cotton seed prior to planting sometimes causes poor seedling emergence. Available information on the effect of substrate moisture and temperature on emergence of phorate-treated cotton seed does not offer an adequate explanation for the results obtained. Therefore the present investigation was undertaken to evaluate the phenomenon more critically.

MATERIALS AND METHODS.—Seed of the cotton variety Deltapine-15 were obtained from a commercial source and acid delinted. Whole firm seed were selected and divided into two lots; one lot was treated with 4 pounds of phorate per 100 pounds of seed by the conventional methyl cellulose-carbon seed treatment method. Briefly, this method consisted of first moistening the acid-delinted seed with a solution of 4% methyl cellulose and then introducing the seed into a jar containing the desired quantity of the carbon dust-phorate mixture and tumbled for 1 hour. The second lot was untreated and was used as the control. The experiments were conducted in constant-temperature tanks as designed by Ranney (1956).

Specially designed pans having a uniform weight were filled with equal weights of air-dried washed river sand and placed in water baths at 67°, 72°, 77°, and 86° F. Twenty-four phorate-treated and 24 untreated seeds were

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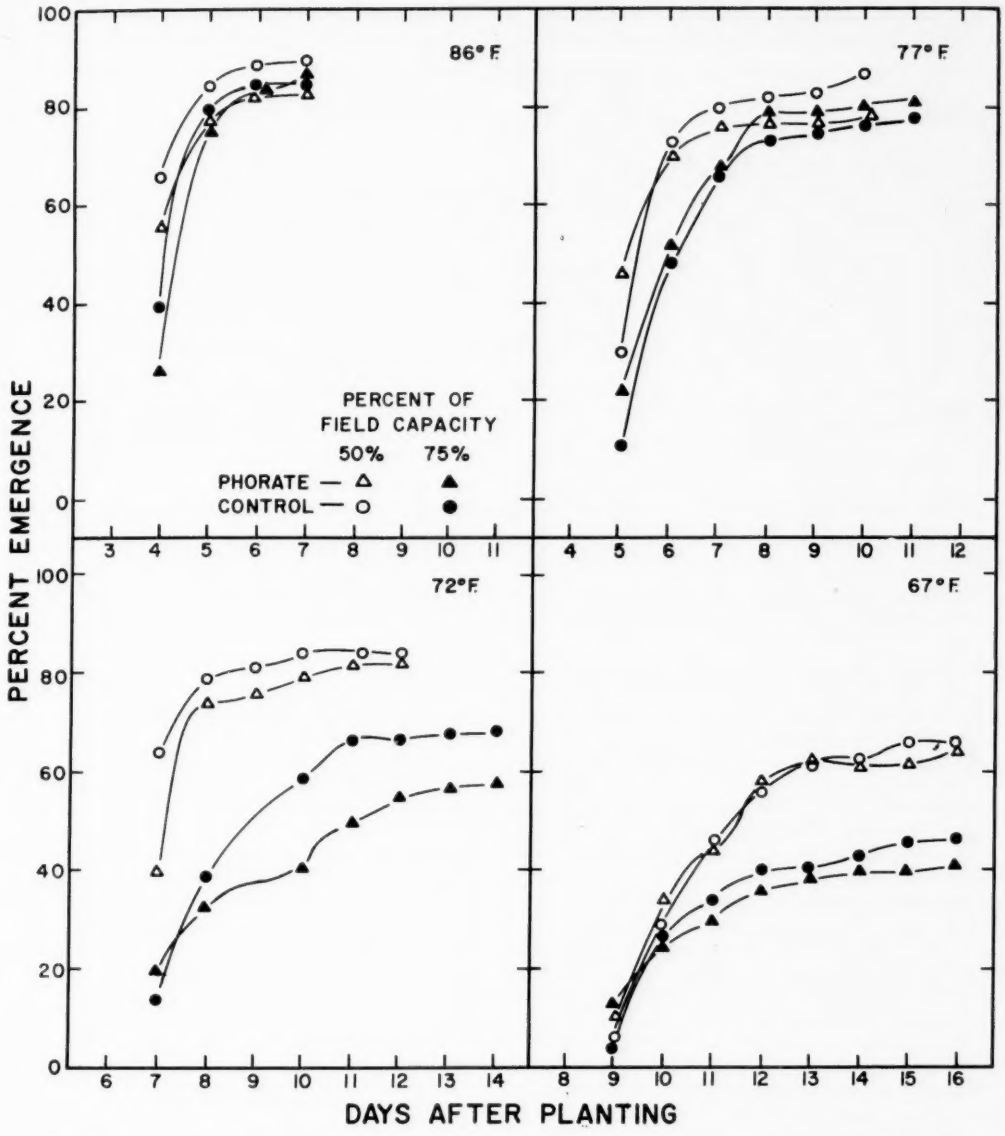

FIG. 1.—Emergence of phorate-treated and untreated Deltapine-15 cotton seed in washed river sand. Seed were obtained from a commercial source, acid-delinted, and treated at the rate of 4 pounds of phorate/100 pounds of seed.

planted in each of four pans at each temperature. Water was added to the sand to produce moisture levels 75% and 50% of field capacity in both the treated and untreated seed at each temperature. The moisture levels in the sand were maintained relatively constant by weighing the pans initially and maintaining the weight at daily intervals by adding water.

A second experiment employing the same variety but a different seed source was performed; however, temperatures of 68° and 80° F. were used and the moisture level was maintained at 50% of field capacity.

RESULTS.—The initial emergence rate of untreated cotton seed at both moisture levels was greater than that of phorate-treated seed at 86° (fig. 1). Also, the higher moisture level (75% of field capacity) caused a slight delay in emergence, but the final total stands were comparable for all treatments. The rate of emergence was slower at 77° than at 86°, but the same general results were obtained.

A sharp decline in the rate and total emergence of seedlings occurred with the phorate-treated seed at 72° F. Again, the untreated seed emerged more rapidly than the

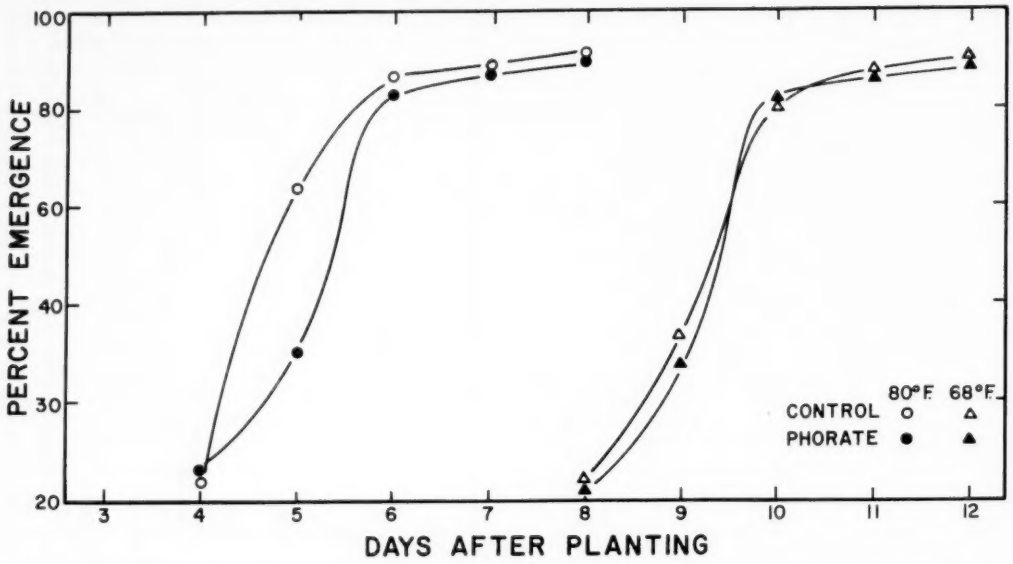

FIG. 2.—Emergence of phorate-treated and untreated Deltapine-15 cotton seed in washed river sand. Seed were obtained from the same commercial source but different lot, acid-deinted and treated at the rate of 4 pounds of phorate/100 pounds of seed.

phorate-treated seed and emergence was reduced and delayed at the higher moisture level. At 67°, the rate of emergence and final stand were even lower than at 72°.

In the second experiment, the rate of emergence of the phorate-treated seed was somewhat slower than that of the untreated seed at 80° F., but the final stands were comparable (fig. 2). At 68°, emergence was delayed 4 days but the rates and total emergence of the phorate-treated and untreated seed were similar. Also, final emergence of the phorate-treated and untreated seed at 68° compared very favorably with that at 80°.

DISCUSSION.—It is commonly known that the cotton plant is thermophyllic and germination of its seed and early seedling growth are very sensitive to low substrate temperatures. Tharp (1960) stated that the average minimum and maximum substrate temperatures for germination and seedling growth are about 60° and 102° F., respectively, whereas the optimum is about 93°. Also, cotton seed contain approximately 15% to 20% oil on a dry-weight basis and, therefore, must have an ample supply of oxygen for proper oil utilization in germination and emergence. These two properties of cotton often present the cotton farmer with serious difficulties in early spring plantings when reduced seedling emergence is found in cold wet soils.

The results presented here show that emergence of cotton seedlings could be adversely affected by a substrate moisture level of 75% of field capacity in the sand used in this study. The excess moisture surrounding the seed undoubtedly limited the gaseous exchange of the respiring seed, thus limiting uptake of oxygen which is required in relatively large amounts for oil utilization. As expected, the reduction in rate of emergence was correlated with decreasing temperatures. The sharp reduction in total emergence of the phorate-treated seed at 72° F. as compared with the untreated seed (fig. 1) was particularly noteworthy because the degree of inhibition due to this insecticide-temperature combination could be observed in this particular source of seed.

Of equal importance was the observation which showed that phorate was without a pronounced adverse effect on seed from a second source (fig. 2). The increased vigor, as measured by increased seedling emergence, in the latter group of seed, was probably due to higher quality. It appears evident, then, that seed quality as well as low temperatures and high soil moisture levels must be considered in evaluating stand losses occurring in phorate-treated cotton seed.

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Fate of DDT and Toxaphene Applied Topically to Susceptible and Resistant Boll Weevils[1]

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Abstract

The metabolism of DDT by susceptible and resistant boll weevils (*Anthonomus grandis* Boh.) treated topically with DDT or DDT plus toxaphene was studied. Little difference was found between strains receiving the same treatments. Extracts of weevils treated with DDT plus toxaphene contained slightly more DDT than those of weevils treated with DDT alone. Poor recovery of the applied DDT was obtained. Tests with C^{14}-labeled DDT indicated that the treated weevils rubbed off much of the toxicant and converted a small quantity to a compound which did not respond to the colorimetric analysis employed.

Resistant weevils treated with toxaphene absorbed less than similarly treated susceptible weevils.

Within the past few years the boll weevil (*Anthonomus grandis* Boh.) has become resistant to toxaphene and some other chlorinated hydrocarbon insecticides in certain areas (Roussel & Clower 1955, 1957, Walker *et al.* 1956). Field tests in which DDT was applied in combination with toxaphene, however, resulted in satisfactory control of resistant boll weevils (Walker & Hanna 1960). Laboratory studies have shown that combinations of toxaphene and DDT exhibit synergistic effects when applied topically to resistant boll weevils (Roussel *et al.* 1959, Brazzel & Lindquist 1960). Roussel *et al.* (1959) found more DDT and less DDE in internal extracts of resistant boll weevils treated with DDT plus toxaphene than in weevils treated with DDT alone.

The work reported here was undertaken to study the metabolism of DDT by laboratory strains of resistant and susceptible boll weevils treated with DDT alone and in combination with toxaphene. The fate of toxaphene alone following application to resistant and susceptible boll weevils also was investigated.

Materials and Methods.—The resistant and susceptible laboratory strains of boll weevils and the rearing techniques employed have been described previously (Brazzel & Lindquist 1960). Technical toxaphene (Entomological Society of America standard, obtained from Nutritional Biochemicals Corp.) and *p,p'*DDT (melting point 108° to 109° C.) were used in these tests. Groups of 30 to 100 weevils, 2 to 5 days old, were treated topically on the dorsal prothoracic suture with 1 μl. of acetone containing the toxicants. The dosages applied were 2.4 μg. of DDT, 2.4 μg. of DDT plus 4.8 μg. of toxaphene, or 4.0 μg. of toxaphene per weevil. Following treatment the weevils were placed in 250-ml. beakers with cloth tops and fed either cotton seedlings or cotton squares (floral buds). The weevils were held at 85° F. and 40% to 60% relative humidity. Fresh food was added daily. At 24, 48, or 72 hours after treatment the dead weevils were discarded. The live weevils were rinsed three times in acetone to remove the toxicant remaining on the cuticle (external extract). These rinsed weevils then were ground in a mortar and pestle with sand and anhydrous sodium sulfate and extracted with carbon tetrachloride, acetone, or *n*-hexane (internal extract) by shaking mechanically for 1 hour.

Table 1.—DDT and DDE recovered from two strains of boll weevils treated with DDT or DDT plus toxaphene.[a]

Insecticide and Dosage in Micrograms	Sacrifice Time (hours)	Micrograms Recovered from External Extracts	Micrograms Recovered from Internal Extracts		Per Cent of DDT Accounted for
		DDT	DDT	DDE	
Susceptible Strain					
DDT, 2.4	24	1.22	0.11	0.02	56
	48[b]	0.63	.68	.00	30
	72	.53	.07	.04	27
DDT, 2.4 plus toxaphene, 4.8	24	.93	.10	.01	43
	48	.65	.13	.06	35
	72	.38	.11	.03	22
Resistant Strain					
DDT, 2.4	24	1.17	0.07	0.03	53
	48	0.72	.05	.02	33
	72	.58	.08	.01	28
DDT, 2.4 plus toxaphene, 4.8	24	1.02	.10	.01	47
	48	0.66	.06	.01	30
	72	.42	.09	.01	22

[a] An average of two to four analyses of groups of 30 to 50 weevils, expressed on a per-weevil basis.
[b] Single analysis of 50 weevils.

A few experiments were run using *p,p'*-C^{14}-ring-labeled DDT with a specific activity of 0.75 millicurie per millimole.

DDT-DDE Analyses.—DDT and DDE (2,2-bis-(*p*-chlorophenyl)-1,1-dichloroethylene; melting point 88° to 89° C.) were analyzed colorimetrically by the method of Schechter *et al.* (1945). DDT and DDE were estimated simultaneously in the same solution by the two-component color system of Knudsen *et al.* (1940). Carbon tetrachloride was the solvent used to obtain the internal extracts. Fat was removed from the internal extracts with acid-celite columns as described by Davidow (1950).

Toxaphene Analyses.—Extracts of toxaphene-treated weevils were analyzed for toxaphene by the titrimetric organic chlorine method developed by Phillips & DeBenedictis (1954) as modified by Rusk (1957). Acetone or *n*-hexane was used to obtain the internal extracts for these analyses.

Results and Discussion.—*DDT.*—The results of the DDT and DDE analyses of internal and external extracts of boll weevils treated with DDT or DDT plus toxaphene show no outstanding differences (table 1). Slightly less DDT remained on the cuticle of DDT-toxaphene-treated

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weevils than on that of DDT-treated weevils for both the resistant and susceptible strains. In most tests slightly more DDT was found in the internal extracts of DDT-toxaphene-treated weevils than in DDT-treated weevils. In both the external and internal extracts there was little difference in the amount of DDT found between strains receiving the same treatment. The amount of DDE in the internal extracts was quite variable, and no general conclusion could be made except that less DDE was found in internal extracts of resistant weevils treated with DDT-toxaphene than in any of the other extracts. These results agree fairly well with the work of Roussel *et al.* (1959), in which field-collected weevils were used, except that these workers found more internal DDT.

The limited number of tests with C^{14}-labeled DDT indicated that DDT was metabolized partially to a compound which did not respond to the colorimetric assay. All of the applied DDT was recovered when based on radioassay. Acetone rinses of both the holding cages and food contained relatively large amounts of radioactivity. Most of this radioactivity was found to be associated with DDT and indicated that the boll weevils rubbed off large amounts of the topically applied DDT.

The question of whether toxaphene synergizes DDT or DDT synergizes toxaphene has not been answered. Previous work has shown that DDT-toxaphene combinations result in synergistic action against resistant boll weevils but not against susceptible ones (Roussel *et al.* 1959, Brazzel & Lindquist 1960). The data presented herein indicate that DDT does not directly cause mortality of either susceptible or resistant boll weevils. This indication is supported by the fact that as much or more DDT was found in internal extracts of the susceptible strain as in the resistant strain following treatment with DDT plus toxaphene. Blum *et al.* (1959) showed that the LD-50 for DDT injected into the boll weevil was approximately 0.5 μg. per insect. In the experiments reported here only about one-fourth of this amount of DDT was found in any of the treated weevils. The low recovery and the tests with C^{14}-labeled DDT indicate that this compound is converted partially to a metabolite which is not detected by the standard colorimetric analysis for DDT.

Toxaphene.—The results of the experiments in which toxaphene was applied to resistant and susceptible weevils are summarized in table 2. Since the analytical method used did not permit a distinction to be made between toxaphene and toxaphene metabolites, all of the results are expressed as toxaphene-equivalents. In these tests the holding cages and food were rinsed with acetone and analyzed. Relatively large amounts of toxaphene-equivalent were found in these rinses. The toxaphene-equivalent in internal extracts of the two strains indicated that the resistant strain absorbed the insecticide more slowly than the susceptible strain. No difference was noted between internal extracts with acetone or *n*-hexane as the solvent.

The results reported here show the need for further metabolism studies on the synergism between DDT and toxaphene or DDT and other cyclodiene insecticides in boll weevils. It would be very interesting to know the action of DDT and toxaphene which results in the synergistic effects of the combination of the two compounds to resistant but not to susceptible boll weevils.

Table 2.—Toxaphene-equivalents found in extracts from two strains of boll weevils topically treated with 4.0 micrograms of the insecticide.[a]

Sacrifice Time (hours)	Micrograms of Toxaphene-Equivalents Recovered from: External Extracts	Internal Extracts	Cage Extracts	Per Cent Recovered
Susceptible Strain				
24	1.2	0.4	1.4	75
48	0.7	.4	1.2	58
72	.7	.8	1.9	85
Resistant Strain				
24	0.9	0.2	1.2	58
48	1.2	.4	2.0	90
72	.7	.3	1.7	68

[a] Four to eight replicates of groups of 50 to 100 weevils, expressed on a per-weevil basis.

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Biology and Control of the Sugarcane Rootstock Weevil on Grain Sorghum in Texas[1]

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Abstract

The sugarcane rootstock weevil, *Anacentrinus deplanatus* Csy., was first observed feeding on sugarcane in Louisiana in 1910 and has become a pest of grain sorghum in Texas. The insect passes the winter in the adult stage in trash near the surface of the soil. The weevil begins to oviposit in grasses during April but soon migrates to grain sorghum. The egg is laid in a small puncture which the female makes in the plant with her beak. Four to 10 days are required for the eggs to hatch. The duration of the larval and pupal stages range from 19 to 32 days and 7 to 16 days, respectively. The longevity of the adult averages 45 days.

The application of either granulated dieldrin or heptachlor to the soil prior to planting resulted in the most effective control of the sugarcane rootstock weevil. Granulated dieldrin and heptachlor applied to the furrow with the seed also controlled the weevil.

The sugarcane rootstock weevil, *Anacentrinus deplanatus* Csy., was first reported in the United States by Holloway (1912). He found the insect feeding on the underground portion of sugarcane plants at Berwick, Louisiana, and at Donna and Brownsville, Texas. Hinds & Osterberger (1932) reported 94% to 100% weevil infestations in sugarcane in Louisiana in 1931 and the weevils were identified by Buchanan (1932) as *A. deplanatus*, and a new species, *A. subnudus* Buch. Bryson (1941) reported *A. deplanatus* attacking grain sorghum in Kansas. The sugarcane rootstock weevil involved in tests conducted at College Station and McGregor, Texas, was identified as *A. deplanatus* Csy. by Miss Rose Ella Warner, U. S. Department of Agriculture. This weevil is now recognized as an economic pest of grain sorghum in Central Texas.

Biological studies were initiated in September, 1959, and were conducted in a laboratory at College Station. This work was performed at a temperature of 78° ± 4° F. and a humidity of approximately 70%. Weevils collected from grain sorghum plants were used to establish a culture. Facilities suitable for observation of the biology of the weevil were improvised by placing adults on a 3-inch section of a sorghum stalk which was placed in a shell vial 25×95 mm. The open end of the vial was covered with black muslin to prevent escape of weevils. The stalk sections were replaced daily. Each stalk section was dissected and examined daily for eggs. The eggs were transferred with a camel's-hair brush to new stalk sections for rearing. Each egg found in the dissected stalk was transferred to a small depression made in the exposed pith of a 3-inch section of a sorghum stalk split longitudinally. The two plant sections were reassembled and sealed at each end with paraffin. Information pertaining to the biology of the insect was obtained by breaking the paraffin seal, separating the two plant parts, and observing the successive stages of development of the insect. Stalk sections were reassembled and resealed with melted paraffin after each daily inspection. Plant sections in vials were replaced as needed. A pair of weevils, obtained from the rearing vials, was transferred to another shell vial containing a fresh sorghum plant section in which the study of the life cycle of the weevil was repeated.

A standard boll weevil medium was used for mass-rearing of the sugarcane rootstock weevil (Brazzel *et al.* 1959). Eggs of the weevil obtained from plant sections in shell vials, as previously described, were transferred directly to the medium in a petri dish. Newly hatched larvae readily entered the medium where development of the insect occurred. Adults that developed in this medium were transferred to vials which contained sections of a sorghum stalk on which they fed and laid fertile eggs.

Results of Biological Studies.—The egg of the sugarcane rootstock weevil is creamy white, oval in shape and averages 0.285 mm. in diameter and 0.475 mm. in length. The female uses her beak to make a small puncture in the plant in which the egg is deposited and concealed. Adults that overwinter in the McGregor area begin oviposition in Johnson grass in April. These weevils later migrate to grain sorghum on which they continue oviposition.

Seventy-four (91%) of the 81 eggs observed in the laboratory hatched. The incubation period ranged from 4 to 10 days and averaged 6.1 days. The minimum and maximum periods required for the development of 69 larvae were 19 and 32 days, respectively, and the average was 24.8 days. Pupation occurred in a special cavity within the pith of the plant section. The maximum and minimum duration of the pupal periods of 61 pupae observed were 16 and 7 days, respectively, and averaged 10.1 days. The average time for development of the sugarcane rootstock weevil from the egg was 41 days and ranged from 30 to 58 days. The female laid an average of 16 eggs.

Distribution and Habits.—The weevil has been found to attack grain sorghum in Cameron, Willacy, Brazos, Burleson, McLennan and Jones Counties in Texas. Overwintering weevils were found in trash near McGregor, McLennan County, on March 22, 1960. The weevils were collected during the growing season from dried leaves and other debris near the base of the host plant, in the soil, on the stalks 3 or 4 inches above the soil and in the sorghum rootstocks. The natural habitat appears to be that area near the base of the plant under detached leaves and in soil around the roots. The weevil feeds on young grain sorghum plants and in the rootstock. Adult injury is noticeable but is not as serious as that caused by the larva.

Weevils when disturbed remain motionless and feign death for several seconds. They are seldom observed by the grower because they are small and have an inconspicuous color pattern. Their habitat also affords protection

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[2] Formerly Research Assistant, and Associate Professor, respectively.

Table 1.—Evaluation of insecticides applied for the control of sugarcane rootstock weevil on grain sorghum.

INSECTICIDE	ACTUAL TOXICANT PER ACRE (LB.)	AVERAGE NUMBER OF WEEVILS[a]
Experiment 1. Mixed with seed before planting		
Phorate	0.5	6.50
Dieldrin	0.5	2.75*
Heptachlor	1.0	4.50*
Untreated		13.25
L.S.D. at 5% level		6.83
Experiment 2. Applied to the surface of the soil		
Dieldrin	1.0	1.00**
	1.5	2.50**
	2.0	2.75**
Heptachlor	1.0	1.50**
	2.5	2.50**
	5.0	2.75**
Phorate	1.0	8.75
	1.5	5.50**
	2.0	12.00
Untreated		12.50
L.S.D. at 5% level		4.35
at 1% level		5.87
Experiment 3. Applied as a sidedressing		
Heptachlor	1.0	12.50
Thimet	1.0	15.25
Dieldrin	1.0	16.50
Untreated		17.75
Experiment 4. Applied as foliar sprays		
One application		
Diazinon	0.26	13.00
Dieldrin	.25	13.25
Dimethoate	1.01	16.25
Endrin	.28	12.75
Sevin	1.54	14.25
Two applications		
Diazinon	0.26 & 0.26	14.50
Dieldrin	.26 & .25	14.25
Dimethoate	1.07 & 1.15	18.75
Endrin	.30 & .30	13.25
Sevin	1.55 & 1.90	17.25
Untreated		19.50

[a] Based on 6 samples of 5 plants each.
* Significantly different at the 5% level from the untreated plots.
** Difference from untreated plots highly significant.

from insecticides applied by commonly used methods.

The feeding of the sugarcane rootstock weevil on grain sorghum often is responsible for lodging of the plants. Exit holes and feeding tunnels made in the plant by this insect also provide conditions favorable for the entrance of charcoal rot, a very serious disease of grain sorghum plants.

CHEMICAL CONTROL.—Four experiments were conducted to evaluate chemical treatments for control of the sugarcane rootstock weevil on grain sorghum (Milo, RS 610) near McGregor. A randomized-block plot design was used in each experiment and the plot size was 20 feet wide (6 rows) and 54.4 feet long. Each treatment and the check were replicated four times.

Each insecticide test consisted of two phases; one involved an application of each insecticide on May 31, 1960, and the other the application of each insecticide to grain sorghum on May 31 and June 14, 1960. The insecticide sprays were applied with a self-propelled machine equipped with No. 3 cone-jet nozzles attached to swivels at the lower ends of drops or extensions mounted at right angles and at 40-inch intervals along the horizontal spray boom. By adjustment of the nozzles on each swivel, the spray material was applied to the lower portions of the plants. The diluted sprays were applied at the rate of 4 gallons per acre and at a pressure of 60 pounds per square inch.

The initial weevil infestation was recorded on May 31, 1960, and other records were taken at weekly intervals until July 6, 1960. A field sample consisted of five grain sorghum plants which were pulled from the soil. The root system and approximately 2 inches of the stalk were examined for weevils. Each plant section, including the main portion of the root system, was cut longitudinally into four approximately equal parts. Only those weevils (larvae, pupae and adults) exposed were recorded as it was impractical to thoroughly examine all the crown and lateral roots.

The effectiveness of granulated formulations of dieldrin, heptachlor, and phorate for control of the sugarcane rootstock weevil was evaluated in Experiment 1. Each insecticide was mixed with the grain sorghum seed as the seed were planted. A planter mounted on the front of a small two-row tractor was used to apply the mixture of seed and insecticide to the furrow. The application of dieldrin at 0.5 pound, and heptachlor at 1 pound of actual toxicant per acre, resulted in the most effective control of this insect (table 1, Expt. 1).

The same equipment used in Experiment 1 was used to apply the insecticides in Experiment 2. The effectiveness of three dosages of granules of each of the insecticides, dieldrin, heptachlor, and phorate applied to the soil surface prior to planting was evaluated for the control of the sugarcane rootstock weevil. The reduction of rootstock weevils in dieldrin- and heptachlor-treated plots was highly significant (table 1, Expt. 2). The reduction obtained with phorate, at 1.5 pounds per acre, also was highly significant. Weevil infestations in plots treated with 1 or 2 pounds of phorate per acre were not significantly less than that found in untreated plots of grain sorghum. Inconsistency of the effectiveness of phorate is inexplainable but it may have occurred because of the overall low weevil infestations in the test areas.

In Experiment 3, granulated dieldrin, heptachlor, and phorate were applied as a sidedressing to the soil surface along each side of the row of sorghum when the plants were approximately 12 inches in height. Insecticides were applied on May 19, with the small tractor-applicator as described above. Grain sorghum plants were sampled and inspected at weekly intervals for weevils from May 31 through July 6 (table 1, Expt. 3). No significant difference was found between the number of weevils found in plots treated with any of the insecticides, nor between any of the treated and untreated plots of grain sorghum. This probably occurred because the weevils apparently had built up to high infestations in the plants prior to the applications of insecticides.

The effectiveness of the application of sprays of Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate), dieldrin, dimethoate, endrin, and Sevin® (1-naphthyl N-methylcarbamate) to grain

sorghum plants for the control of the sugarcane rootstock weevil were evaluated in Experiment 4. Each test consisted of two phases; one involved an application of each insecticide on May 31 and the other concerned an application of each insecticide to grain sorghum on May 31, and June 14. Records of the number of weevils in five plants from each plot were made at weekly intervals from May 31 to July 6 (table 1, Expt. 4). No significant difference was found between the number of weevils in grain sorghum plants treated with any of the insecticides, nor between any of the treated and untreated plots of grain sorghum.

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Description and Results of a New Greenhouse Technique for Evaluating Tolerance of Small Grains to the Greenbug[1]

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ABSTRACT

Test varieties are grown in flats and infested with greenbugs (*Toxoptera graminum* (Rond.)) at emergence. The insects are not caged but are allowed to migrate freely from plant to plant to simulate infestation under field conditions. Plants destroyed within 2 weeks are considered to be susceptible while others are rated as tolerant. Eight thousand varieties were evaluated. Five *Triticum vulgare* and 14 T. *durum* varieties have shown a high degree of tolerance.

The greenbug (*Toxoptera graminum* (Rond.)) is considered the most destructive pest of small grains in the Central and Southwestern States. During years of severe infestation, losses estimated at more than 50 million bushels of grain have occurred. These losses have been reduced in recent years by the application of insecticides; however, in many areas where yields are low the expense of this method of control prohibits its use. Since cultural practices and natural enemies have not resulted in successful control, the development of other methods has become necessary. The most promising of these is growing of resistant varieties. Studies were therefore initiated in Oklahoma in 1947 to find greenbug-resistant germ plasm for use in small grain improvement.

In 1947 Dahms *et al.* (1955) developed a technique for screening small grains for resistance to the insect. It involved caging a known number of greenbugs on several varieties grown in each pot. In this manner data such as plant tolerance, greenbug preference, and fecundity were determined. However, obtaining a measurement of tolerance was often difficult since the greenbugs, which increased very rapidly, destroyed the tolerant as well as the susceptible lines within a few days. Chada (1959) described a method for testing the resistance of small grains to greenbugs in a controlled-environment insectary in which the insects were confined to test varieties growing in flats covered with plastic cages. Since the greenbugs were caged on the plants, this method had the same disadvantage as the one described by Dahms in that it did not simulate field conditions. A new greenhouse technique developed to evaluate small grain varieties for tolerance to the greenbug and some results obtained with its use are reported in this paper.

DESCRIPTION OF TECHNIQUE.—Greenbug cultures were maintained on the susceptible variety Ward barley. The variety of seed to be evaluated is planted in pots in a circular pattern, in order to increase ventilation and reduce mildew. The soil is a sandy loam fairly high in organic material. The seed is covered with a 1-inch layer of sand to prevent excessive evaporation. After planting, the pots are placed in metal pans containing 2 to 3 inches of water until the soil is well soaked. The water is then siphoned from the pans.

Before the plants emerge they are covered with a cage inserted into the soil in each pot to protect them from premature infestation. The cages are constructed of transparent, cellulose nitrate plastic tubes 12 inches in height, 5 inches in diameter, and 0.02 inch in thickness (fig. 1).

After 14 days the plants are infested by introducing approximately 100 greenbugs into each pot. To combat mildew during warm, moist periods the plants are dusted with sulphur before infestation. After a feeding period of 10 to 15 days these greenbug cultures are ready for use. Fifty galvanized metal flats $14 \times 20 \times 3\frac{3}{4}$ inches are used in conducting each test. The rows in which the seeds are placed are made with a "row-maker" which fits into the flat (fig. 2). This apparatus consists of two pieces of galvanized sheet metal welded together, the bottom having ridges which when pressed into the soil produce 10 evenly spaced rows of uniform depth. The top is equipped with two metal handles. Approximately 20 seeds of each variety are planted in a row and thinned to 10 plants at emergence. A row containing a susceptible variety is planted in each flat, and a resistant variety is included in every fifth flat. Each row is staked and labeled (fig. 3).

The plants are infested at emergence by uniformly sprinkling approximately 2,000 greenbugs over each flat. The aphids are brushed from the culture plants onto a sheet of paper to facilitate estimating their population. Another test may be started soon after the first infesta-

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FIG. 1.—Culture room showing the method of rearing greenbugs; *a*, Ward seedlings prior to infesting; *b*, infested cultures; *c*, water pans.

tion so that plants in different stages of development may be observed simultaneously.

The number of days the plant lives is used as a measure of tolerance, and plants are rated periodically using the system described by Dahms *et al.* (1955). Any plants that approach the tolerance of the resistant check are transplanted to pots and allowed to mature. Seeds from these plants are again tested in turn to assure that the line retains resistance.

Plant breeders then cross resistant lines with varieties having desirable agronomic characteristics and the segregating generations are again screened for greenbug tolerance. Hybrids with tolerance and their parents are then grown in the field and infested with greenbugs cultured in the greenhouse. This is accomplished by scattering infested barley cuttings evenly over the plots. With this technique winter hardiness, drought resistance, and other desirable agronomic characteristics may be selected, in addition to greenbug tolerance.

RESULTS.—Approximately 8,000 lines of the World

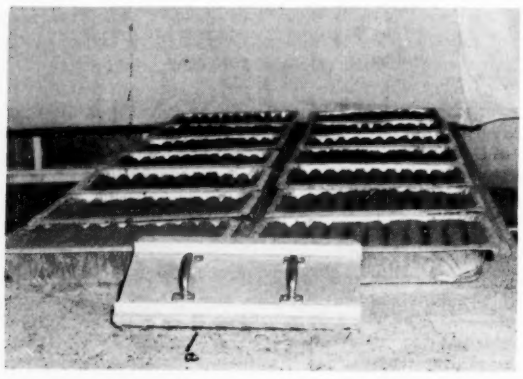

FIG. 2.—Methods of preparing flats for planting; *a*, ridged bottom of rowmaker.

Table 1.—Wheat varieties of the World Wheat Collection possessing a high degree of tolerance to the greenbug.

VARIETY	SOURCE	C. I. OR P. I. NO.
		T. vulgare type
Dickinson Sel. 28-A	N. Dakota	3707
Unnamed	Russia	9058
F.W. 806-X-7	Georgia	13125
Timstein×(KV. Mqo. ×Kv.-Tq.)	Kansas	13287
Bagdad	Arabia	2202
		T. durum type
Unnamed	Egypt	7261-15
		7501
		7503
		7508
		7520
		7523
		7526
	Ethiopia	7833
	Arabia	145720-10
Collita tipo 187	Portugal	192064
Beladi	Egypt	57662-1
Unnamed	Ethiopia	194025
		194043
		195694

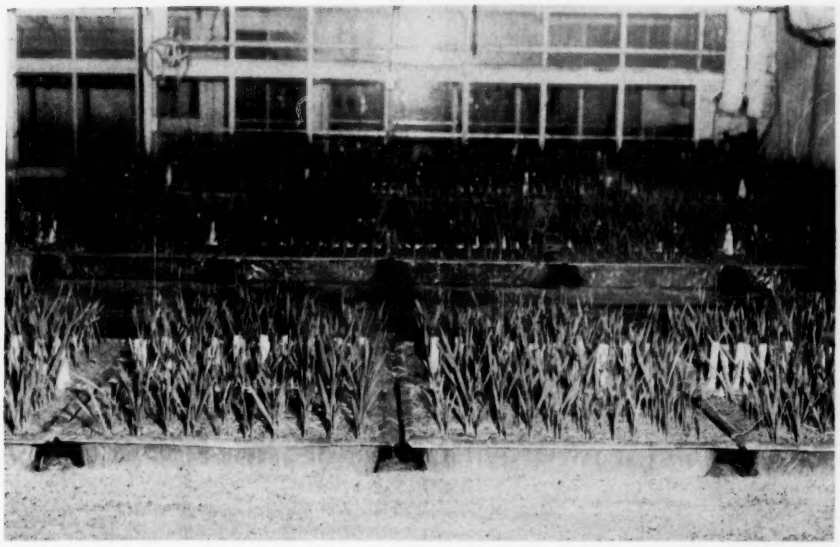

FIG. 3.—Screening room showing a series of barley lines being screened for resistance.

Wheat Collection have been screened by this method in a period of 4 years. Five *Triticum vulgare* and 14 *T. durum* lines have been found that possessed a high degree of tolerance and are listed in table 1. Dickinson Sel. 28-A (C.I. 3707) and an unnamed variety (C.I. 9058) have been successfully crossed with recommended wheat varieties and selections have been made from the resultant hybrids.

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Some Factors Influencing Light Trap Collections[1]

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ABSTRACT

Field tests of survey-type electric insect traps conducted at College Station and Brownsville, Texas during 1959 showed that cylindrical enclosures of ¼-inch mesh hardware cloth reduced the catches of large species of insects by a factor of 70% or more, while reducing the catches of smaller species of insects, comparable in size to adult pink bollworms (*Pectinophora gossypiella* (Saund.)), by a factor of 20% to 45%. Tests with artificial windbreaks showed that catches of all species were increased by shielding the traps from the effects of prevailing winds.

The extensive use of light traps to survey the distribution of the pink bollworm (*Pectinophora gossypiella* (Saund.)) in Arizona in conjunction with an eradication program begun there in 1958 has brought forth several problems concerning the most effective methods of installing and operating these traps. Two of the problems, namely, (1) the influence of enclosing the traps with hardware cloth cylinders and (2) the influence of wind protectors or "windbreaks" upon trap efficiency, were studied during the summer of 1959 at College Station and Brownsville, Texas.

INFLUENCE OF SCREEN COVERINGS.—The time required for examination of collections increases and the destruction of many of the pink bollworm specimens results when larger species of insects are allowed to enter light traps during operation. Tests were conducted to determine if enclosing a trap with a cylinder of ¼-inch mesh hardware cloth (figs. 1 and 2) would eliminate these larger species without drastically influencing the catch of the smaller species.

Methods.—The general procedure involved comparison of the nightly collections of insects in traps covered with the cylinders with those in traps without the cylinders. Traps equipped with one 15-watt black light fluorescent lamp (General Electric Co. Type F15/T8/BL)[4] and

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[4] Mention of trade names of specific equipment in this publication is made for purpose of identification and does not imply endorsement by the United States Department of Agriculture over similar equipment not mentioned.

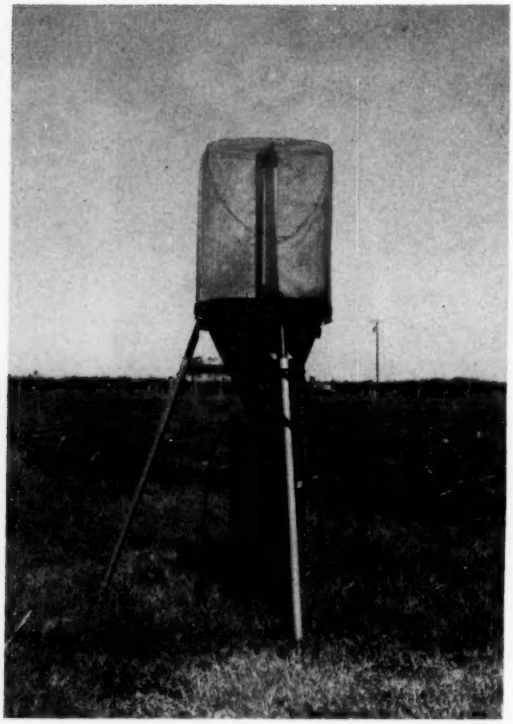

FIG. 1.—Black light survey trap equipped with ¼-inch mesh hardware cloth cover.

others with three 2-watt argon glow lamps (General Electric Co. Type AR-1) were used for the comparisons.

The tests with the black light traps conducted at College Station were divided into two series. The first of these was carried out from July 18 through August 14 and the second from September 3 through 24. In the first series two black light traps were operated approximately 70 feet apart, while in the second they were placed 55 feet apart. Three rotation systems were tried during the course of the experiments. These included alternation of the screen cover between the two traps nightly, alternation of the trap positions nightly, and a combination of both rotations in which trap positions were alternated every 4 nights and the cover was alternated nightly. None of these appeared to have any significant effect on the results. Black light fluorescent lamps producing nearly equal measured outputs in a narrow wave band centered at 365 millimicrons (in the near ultraviolet region of the spectrum) were selected for use in the traps.

During the first half of the test the nightly collections were weighed and the volume measured. This procedure was modified during the latter half of the first series by the addition of counts of cotton bollworm moths (*Heliothis zea* (Boddie)) and microlepidoptera of adult pink bollworm size. The latter were determined by sifting the collections through a #6 screen (6 holes per inch) and then through a #8 screen (8 holes per inch). The moths retained by the latter screen were approximately the size of pink bollworm adults. In the second series counts of cabbage looper adults (*Trichoplusia ni* (Hübner)) which occurred in considerable numbers during this period were added.

Tests of the argon traps were made at College Station and near Brownsville using the combined rotation of trap position and screen cover described above. The College Station tests were carried out from August 18 through September 2. The measured outputs of 365 millimicrons radiation from each of the argon lamps selected for use in the traps was approximately equal. The traps were placed approximately 55 feet apart adjacent to a cotton field.

At Brownsville the tests were conducted from July 28 through September 11 near a cotton field infested with pink bollworms. The traps were placed approximately 30 feet apart and the screen cover was alternated nightly. Records of the number of male and female adult pink bollworms collected were made as well as the weight and volume of other insects.

Results and Discussion.—The results, summarized in table 1, indicate a reduction in all categories when the screen coverings were used. The reductions were most apparent for the total catch, as indicated by the weight of the collections, and for the two larger species of moths recorded, the cotton bollworm and cabbage looper. These reductions were 70% or greater from the collections in the unscreened traps. The volume measurements showed similar trends, although they were more variable. These latter data are not included as it was believed that the weight method furnished the more accurate measurement.

The collections of the smaller moths of pink bollworm size were reduced only 20% to 45%. The two sexes of the pink bollworm followed the same trend of reduction as the total pink bollworm population at Brownsville. At Brownsville, collection in the screen trap relative to the unscreened one varied considerably; however, this was not so apparent at College Station.

The results of these tests indicate that the use of ¼-inch mesh hardware cloth covers on light traps operated for pink bollworm survey purposes is warranted only when larger species of insects are present in sufficient

FIG. 2.—Argon survey trap equipped with ¼-inch hardware cloth cover.

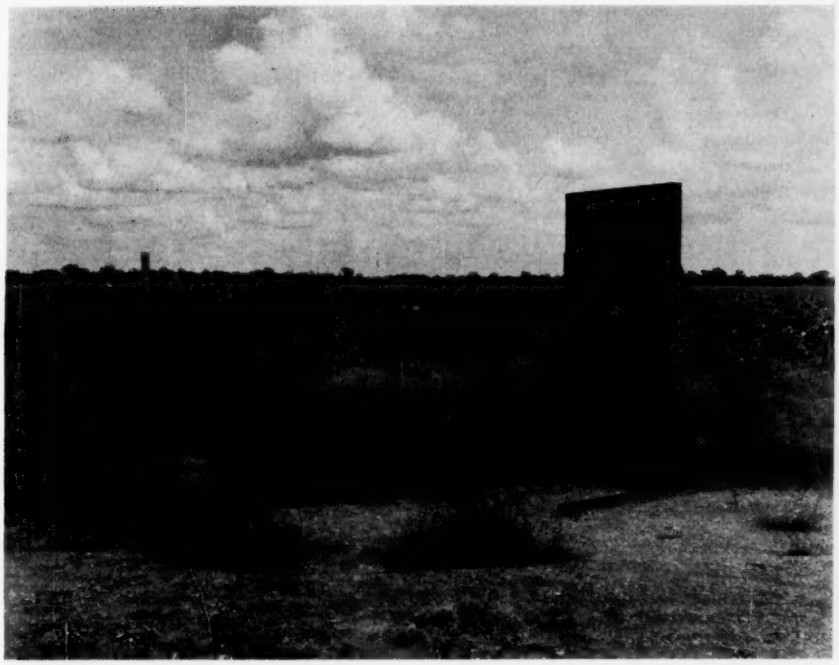

FIG. 3.—Test site at College Station for determining effect of windbreak on light trap insect collection.

numbers to make examination of the collections extremely difficult.

INFLUENCE OF WINDBREAKS.—It has been noted in previous work on insect collection by light traps that larger collections were taken in traps located behind windbreaks, such as trees, bushes and buildings, than in similar traps in exposed locations. Tests were carried out in College Station and Brownsville to obtain more precise data on this phenomenon.

Methods.—At each location two light traps were placed some distance apart (40 feet at College Station, 20 feet at Brownsville) near cotton fields. An artificial windbreak consisting of a 4-×8-ft. piece of plywood painted with flat black paint was placed by one of the traps. Placement was such that the trap was shielded from the prevailing southeasterly winds. The other trap was exposed to the wind (fig. 3). A totalizing anemometer was operated between the two traps at trap height. The tests at College Station utilized two unidirectional 15-watt black light traps, while the traps at Brownsville were of the standard argon glow lamp type and were identical to the argon traps being used in Arizona for pink bollworm detection. The argon trap was placed approximately 12 inches from the front side of the windbreak. At College Station the trap locations were alternated every 3 nights; that is, for 3 nights Trap No. 1 was operated behind the windbreak and No. 2 in the open, then for 3 nights No. 2 was behind the windbreak and No. 1 in the open. This procedure was repeated throughout the test. At Brownsville the positions were not changed. The weight and volume of the nightly collections were recorded. The collections of cotton bollworm adults at College Station and pink bollworm adults at Brownsville were also recorded. The College Station tests were started July 17 and continued for a total of 30 nights. Those at Brownsville were run for 15 nights between August 11 and September 10.

Table 1.—Influence of ¼-inch hardware cloth screen on insect collections by survey light traps.

TEST	NUMBER DAYS RECORDED	NO SCREEN: MEAN PER DAY	SCREEN: MEAN PER DAY	SCREEN: REDUCTION WITH SCREEN (%)
Weight of collections (grams)				
BL Series I	26	204.5	49.2	75.9
BL Series II	16	300.7	76.7	74.5
Argon—College Station	15	93.3	24.5	73.7
Argon—Brownsville	22	145.2	32.8	77.4
Number moths pink bollworm size				
BL Series I	8	571.1	312.0	45.4
BL Series II	16	515.8	361.9	29.8
Argon—College Station	15	197.8	155.4	21.4
Argon—Brownsville[a]	22	138.9	100.5	27.7
Number cotton bollworm moths				
BL Series I	16	84.5	3.0	96.4
BL Series II	16	20.8	4.6	77.9
Argon—College Station	15	24.3	5.7	76.5
Number cabbage looper moths				
BL Series II	16	40.9	12.4	69.7
Argon—College Station	12	79.9	19.7	75.3

[a] Actual pink bollworm moths.

Table 2.—Influence of windbreak on light trap collections —College Station, Texas.

Date	Mean Wind Velocity (m.p.h.)	Windbreak: Total Catch Weight (gm.)	Windbreak: Cotton Bollworm No.	No Windbreak: Total Catch Weight (gm.)	No Windbreak: Total Catch Difference from Windbreak (%)	No Windbreak: Cotton Bollworm No.	No Windbreak: Cotton Bollworm Difference from Windbreak (%)
July 17	3.1	66	—	32	− 51.5	—	
18	7.4	50	—	44	− 12.0	—	
19	6.0	22	—	28	+ 27.3	—	
20	2.1	22	—	14	− 36.4	—	
21	2.8	97	—	32	− 67.0	—	
22	3.5	86	—	69	− 19.8	—	
23	3.6	130	—	82	− 36.9	—	
24	6.6[a]	122	—	36	− 70.5	—	
26	11.4[a]	2	—	22	+500.0	—	
27	7.4	170	—	150	− 11.8	—	
28	7.3	150	168	102	− 32.0	86	− 48.8
29	3.0	134	130	92	− 31.3	118	− 9.2
30	2.0	136	124	99	− 27.2	70	− 43.6
31	0.8	204	74	160	− 21.6	42	− 43.2
Aug. 1	1.1	177	90	144	− 18.6	66	− 26.7
2	4.0	202	204	202	0.0	204	0.0
3	5.0	153	109	182	+ 19.0	184	+ 68.8
4	5.1	86	122	107	+ 24.4	128	+ 4.9
5	3.2	124	192	128	+ 3.2	—	
6	4.8	293	458	176	− 39.9	260	− 43.2
7	2.7	287	490	234	− 18.5	377	− 23.0
8	3.5	143	344	150	+ 4.9	284	− 17.4
9	1.7[a]	141	348	101	− 28.4	304	− 12.6
10	2.8[a]	207	768	77	− 82.1	264	− 65.6
11	4.3[a]	158	444	69	− 56.3	151	− 66.0
12	1.6	131	64	94	− 28.2	140	+118.8
13	2.1	121	298	68	− 43.8	102	− 65.8
14	4.9	84	106	32	− 61.9	36	− 66.0
15	6.6	42	63	13	− 69.0	33	− 47.6
16	3.5	40	32	35	− 12.5	27	− 15.6
Mean		126.0	231.4	92.5	− 26.6	141.1	− 39.0

[a] Wind from northerly direction into front of windbreak.

Table 3.—Influence of windbreak on light trap collections —Brownsville, Texas.

Date	Mean Wind Velocity (m.p.h.)	Windbreak: Total Catch Weight (gm.)	Windbreak: Pink Bollworm No.	No Windbreak: Total Catch Weight (gm.)	No Windbreak: Total Catch Difference from Windbreak (%)	No Windbreak: Pink Bollworm No.	No Windbreak: Pink Bollworm Difference from Windbreak (%)
Aug. 11	1.9	33	67	26	−20.0	36	−46.3
12	1.9	32	78	12	−61.9	38	−51.3
13	1.6	31	93	8	−74.0	40	−57.0
14	2.1	24	76	9	−62.8	16	−78.9
18	2.6	9	45	4	−57.8	10	−77.8
19	2.2	30	109	10	−66.0	25	−77.1
20	1.0	21	96	13	−38.1	64	−33.3
21	0.9	16	124	14	− 9.9	86	−30.7
25	2.8	36	152	27	−24.4	39	−74.3
Sept. 1	4.4	14	40	6	−61.0	91	+56.0
2	5.4	9	48	4	−60.2	10	−79.2
3	2.9	10	36	4	−63.5	76	+52.6
4	2.3	19	48	6	−70.4	44	− 8.3
9	1.4	15	40	4	−71.3	26	−35.0
10	0.7	18	40	7	−60.8	20	−50.0
Mean		21.1	72.8	10.3	−51.4	41.4	−43.1

Results and Discussion.—The results of these tests are summarized in tables 2 and 3 and show that the collections of all three categories were reduced when the traps were operated in the exposed location. The reductions were more conspicuous at Brownsville than at College Station, which was possibly the result of the difference in trap types and procedure as well as environmental conditions. It appears that, in general, the cotton bollworm adults were affected more than the mass of other insects, while the pink bollworm moths were affected the least, although the differences may not be significant.

Northerly winds were encountered on 6 of the nights the traps were operated at College Station and on none of these at Brownsville. These data were taken from U. S. Weather Bureau records at Easterwood Airport, located a few miles from the College Station site, and the Brownsville International Airport, located approximately 3 miles from the Brownsville site. Comparisons between the wind conditions at the experimental sites and the weather stations showed rather close correlation. It might be anticipated that with the windbreaks located on the southeast side of the traps northerly winds would nullify the differences between the two trap exposures. However, on 4 of the 5 nights that northerly winds occurred at College Station this was not true. It is possible that a quonset hut situated to the north of the screened trap may have had some influence on this effect. More detailed information on wind movement and velocity than was obtained would be necessary for an analysis of the influence of these factors.

The results of these tests indicate that light traps shielded from the prevailing winds are more efficient in collecting insects than traps in more exposed locations. It appears that the windbreaks may also increase the collections when the winds are from the opposite direction. Of course, if the windbreak is too extensive it will undoubtedly reduce the collections in nearby traps by the shielding of light from some of the insects in the area.

Some Effects of Toxaphene-DDT and Calcium Arsenate on Growth and Development of Cotton[1]

LAMAR C. BROWN, CHARLES LINCOLN, R. E. FRANS, and B. A. WADDLE[2]

ABSTRACT

Treatments of toxaphene-DDT and calcium arsenate were begun when cotton was at the cotyledonary stage of development and were terminated after 7, 12, and 16 weekly applications. All insecticide treatments significantly reduced plant height, with suppression being greater in plots treated with calcium arsenate for 16 weeks during the growing season.

A significantly greater number of bolls were produced in plots treated with toxaphene-DDT for 12 and 16 weeks; however, any advantage was offset by a concurrent reduction in boll size and weight, and yield was not affected. Sixteen weekly applications of calcium arsenate resulted in a significant reduction in yield, seed weight, lint index, boll weight, dry weight of plants, and boll production. Other calcium arsenate treatments affected only the height of cotton plants.

None of the insecticide treatments affected earliness, seeds per boll, per cent lint, per cent shed, and germination.

The authors and others have observed symptoms of phytotoxicity such as leaf discoloration and/or varying amounts of defoliation in cotton field plots treated with toxaphene-DDT and stunted plants and/or discolored leaves in plots treated with calcium arsenate. Data showed no yield reduction, however, in plots wherein phytotoxic symptoms were observed. Similar observations have been reported in the literature; *e.g.*, Joyce (1954) reported spotting of leaves caused by toxaphene treatments but noted no prolonged effect on cotton plants and no subsequent deleterious effect on yield. Possible adverse effects of insecticides on various components of the cotton plant, however, might not be easily discernible under some conditions; *e.g.*, any damage caused by insecticide treatments could be compensated in part by the protection from insects afforded by the insecticides. Recent evidence, however, indicates an effect of certain insecticides on cotton aside from the consideration of insects *per se*. Work by Hacskaylo & Scales (1959) showed that Guthion, Guthion-DDT, and dieldrin-DDT formulations influenced the growth and development of cotton under insect-free conditions in the greenhouse. Also, preliminary greenhouse work by the authors indicated a trend toward a reduction in height due to calcium arsenate treatments. In addition, it has been a common observation that cotton treated with toxaphene-DDT often appears to mature earlier in the field. Open cotton is naturally more visible because of fewer leaves due to defoliation, and possibly plants partially defoliated by insecticides might create an "illusion" of early maturity as compared with undefoliated cotton. However, fruiting often ceases earlier in stunted and/or damaged cotton, and plants subsequently may have a greater percentage of bolls open earlier in the season than undamaged cotton. Observations of apparent plant response to toxaphene-DDT and calcium arsenate suggested the need for more conclusive experimental evidence on the effects of these insecticides on cotton. Since it was deemed advisable to conduct the work in the field, the experiment reported herein was conducted at the University of Arkansas Experiment Station, Fayetteville, where naturally occurring populations of insects harmful to cotton are relatively low.

METHODS.—The experiment was conducted in the field in 1959. The design utilized was a randomized block with four replications. Plants of the cotton variety Plains (*G. hirsutum* L.) were spaced about 6 inches in the row and each plot consisted of three rows, 50 feet long. Data were taken from only the center row. The insecticide treatments consisted of a 2:1 mixture of toxaphene-DDT at a rate of 2 pounds of toxaphene and 1 pound of DDT in 10 gallons of water per acre, and calcium arsenate (high suspension) at a rate of 10 pounds in 10 gallons of water per acre. The insecticides were mixed with water just prior to application with a tractor-mounted sprayer. The tractor also moved through the check plots so that any damage caused by ground machinery would be essentially the same in all plots. Applications were made when wind velocity was nil and therefore no drift problems were encountered.

Treatment designations were as follows: treatments 1, 3, and 5 utilized toxaphene-DDT, treatments 2, 4, and 6 calcium arsenate, and treatment 7 no insecticide (check). The insecticide treatments were begun when the cotton plants were at the cotyledonary stage of development and were terminated at different intervals during the growing season as follows: Treatments 1 and 2 represent 7 weekly insecticide applications terminated when plants began flowering; treatments 3 and 4 were discontinued after 12 weekly insecticide applications, at which time the oldest bolls were about 35 days old; and, treatments 5 and 6 were terminated after 16 weekly insecticide applications, when numerous open bolls were present.

The plots were checked several times each week and no infestations of harmful insects were detected during the growing season. Approximately 1 week before the experiment was terminated a minor outbreak of the cotton leafworm (*Alabama argillacea* (Hbn.)) was noted in cotton adjacent to the experimental plots. As a precaution the experimental plots were treated with malathion-DDT. The leafworm was controlled in adjacent cotton and no damage was detected in the experimental plots.

RESULTS.—*Height.*—One of the most pronounced effects of the insecticide treatments on cotton was a reduction in height (table 1). All insecticide treatments reduced height significantly as compared with the check, with the reduction being greater in plots treated with

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Table 1.—Effect of toxaphene-DDT and calcium arsenate on internode length, number of internodes, and height of cotton treated at different intervals during the growing season.[x]

Treatment No.	No. of Weekly Applications	Internode Length (cm.)	No. of Internodes	Height (cm.)
		Check		
7	None	7.1[e]	15.3[bcd]	108.6[e]
		Toxaphene-DDT		
1	7	6.6[b]	15.5[cd]	102.2[d]
3	12	6.4[b]	15.7[d]	100.1[cd]
5	16	6.4[b]	15.1[bc]	96.9[bc]
		Calcium arsenate		
2	7	6.7[b]	14.9[b]	100.1[cd]
4	12	6.6[b]	14.4[a]	94.4[b]
6	16	5.9[a]	14.9[b]	88.2[a]

[x] Any two means with the same letter in superscripts within a column are not significantly different from each other at the 5% level.

calcium arsenate. In general, plant growth was retarded to a greater degree in plots treated for longer intervals during the growing season. Internode length was reduced significantly by all insecticide treatments; number of internodes was reduced only by Treatment 4, 12 weekly applications of calcium arsenate.

Weekly measurements of height of plants were begun 2 weeks after the first insecticide treatments and were continued throughout the remainder of the season. All insecticide treatments reduced height throughout the growing season as compared with the check (fig. 1). However, the suppression of growth was magnified only so long as the insecticide treatments were continued; *i.e.*, plants in any given plot wherein the insecticide treatment was terminated showed an increase in growth over that of plants in plots wherein the treatments were continued. In no instance, however, did the height of treated plants equal that of the untreated plants.

Yield, Boll Components, and Germination.—Treatment 6, 16 weekly applications of calcium arsenate, significantly reduced the yield of seed cotton (table 2). Other treatments did not affect yield. Seed weight, lint index, and boll weight were reduced significantly by Treatment

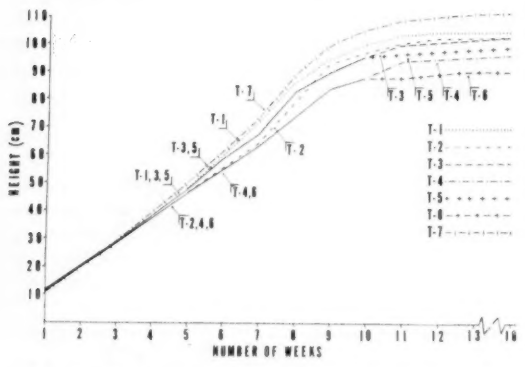

FIG. 1.—Height of cotton as influenced by treatments of toxaphene-DDT and calcium arsenate.

Table 2.—Yield, boll components, and germination as affected by treatments of toxaphene-DDT and calcium arsenate.[x]

Treatment No.	No. of Weekly Applications	Yield (lb.)	Seed[y] Index	Lint[z] Index	Boll Weight (gm.)	Seeds per Boll	Lint (%)	Germination (%)
				Check				
7	None	7.70[b]	12.3[c]	8.0[c]	7.35[b]	36.0	38.8	83
				Toxaphene-DDT				
1	7	7.94[b]	12.2[c]	7.8[bc]	7.22[b]	36.0	39.3	83
3	12	7.85[b]	11.5[a]	7.2[a]	6.82[a]	36.0	38.8	82
5	16	7.92[b]	11.5[a]	7.2[a]	6.86[a]	36.0	38.8	78
				Calcium arsenate				
2	7	7.65[b]	12.2[c]	8.0[c]	7.16[b]	35.5	39.5	82
4	12	7.96[b]	12.4[c]	8.2[c]	7.31[b]	35.3	39.5	85
6	16	6.62[a]	11.9[b]	7.5[ba]	6.73[a]	34.9	39.0	82
						N.S.	N.S.	N.S.

[x] Any two means with the same letter in superscripts within a column are not significantly different from each other at the 5% level.
[y] Weight in grams of 100 ginned seeds.
[z] Weight in grams of the fiber from 100 ginned seeds.

6 of the calcium arsenate series and Treatments 3 and 5 of the toxaphene-DDT series; Treatments 1, 2, and 4 had no effect on boll components. The insecticide treatments had no effect on seeds per boll, per cent lint, or germination.

Dry Weight and Boll Production.—Dry weight of plants was significantly reduced in plots treated for 16 consecutive weeks with calcium arsenate (table 3); there were no significant differences among the remaining treatments with respect to dry weight. Boll production was significantly reduced by Treatment 6 whereas Treatments 3 and 5, of the toxaphene-DDT series, resulted in the production of a significantly greater number of bolls. There were no significant differences among treatments with respect to per cent of bolls shed or earliness.

DISCUSSION.—The most obvious effect attributed to the insecticides used in this study was a significant reduction in height. However, the effect of the insecticides on plant height was temporary and height increased in plots wherein treatments were terminated as compared with plots receiving continuing treatments of insecticides. It may be questionable whether a reduction in height *per se* would prove deleterious to cotton under some conditions (Frizzell *et al.* 1960); however, other components were

Table 3.—Dry weight, number of bolls produced, per cent shed, and earliness as affected by treatments of toxaphene-DDT and calcium arsenate.[x]

Treatment No.	No. of Weekly Applications	Dry Weight per Plant (gm.)	No. of Bolls Produced (%)	Shed (%)	Earliness[y]
			Check		
7	None	36.5[b]	100[c]	52.8	54.0
			Toxaphene-DDT		
1	7	31.5[b]	103[c]	50.5	59.5
3	12	33.2	108[b]	49.5	68.8
5	16	33.2[b]	109[b]	48.3	57.0
			Calcium arsenate		
2	7	31.7[b]	100[c]	52.3	51.5
4	12	31.7[b]	102[c]	50.5	59.7
6	16	25.2[a]	92[a]	54.5	63.3
				N.S.	N.S.

[x] Any two means with the same letter in superscripts within a column are not significantly different from each other at the 5% level.
[y] Per cent of seed cotton picked at first harvest.

drastically affected by certain treatments. Sixteen weekly applications of calcium arsenate resulted in a significant reduction in yield, seed weight, lint index, boll weight, dry weight of plants, and boll production. Other than plant height, however, neither the 7 nor the 12 weekly application series of calcium arsenate affected the components measured in this study.

The 12 and 16 weekly application series of toxaphene-DDT resulted in the production of a significantly greater number of bolls. However, any apparent advantage as a result of increased boll production due to these treatments was offset by a concurrent reduction in boll size and weight, and yield was not affected.

Symptoms of insecticide damage to plants, such as leaf discoloration and defoliation, were not discernible during the experiment and there was no apparent damage to cotton from the use of ground machinery. The absence of phytotoxic symptoms other than stunted growth did not permit a comparison of so-called "visual earliness" with actual data. The results of this study, however, showed no significant differences among treatments with respect to earliness.

Insect infestations were not considered sufficient during the growing season to affect the results of this experiment. Also, the lack of differences among treatments with respect to per cent of boll and square shed, together with a lack of insect infestation, indicates that the plant responses herein reported were caused primarily by the insecticides utilized in this study.

Work by Hacskaylo & Scales (1959) showed that dieldrin-DDT retarded flower formation, boll set, and plant growth whereas low rates of Guthion resulted in increased boll production. Also, all insecticides used in their experiment reduced boll size. It is also well known that DDT may prove to be injurious to various species of plants at high concentrations and cause plant stimulation at low concentrations (Chapman & Allen 1948, Allen & Casida 1951). Information to date strongly suggests the possibility that insecticides *per se* do affect the growth and development of cotton, and additional work in this field is suggested.

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Inheritance of Resistance to Endrin in the Boll Weevil[1]

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ABSTRACT

Genetic aspects of endrin resistance in the boll weevil, *Anthonomus grandis* Boh., were investigated by subjecting the following strains and crosses to scalar doses of endrin: (a) endrin-resistant and -susceptible strains and (b) the F_1 and F_2 progeny of reciprocal crosses of these strains.

The results indicate that endrin resistance was genetically controlled in these boll weevil strains. From the data at hand, the indications are that endrin resistance in these strains and crosses was controlled by more than one pair of additive autosomal genes.

A review of the literature relative to genetics of insecticide resistance in insects was presented by Crow (1957). This author as well as Milani (1957) and Hoskins & Gordon (1956) pointed out some of the difficulties of determination of mode of inheritance of resistance with dosage-mortality lines. The development of resistance in the boll weevil, *Anthonomus grandis* Boh., to certain chlorinated hydrocarbon insecticides was reported by Roussel & Clower (1955).

The results reported herein were obtained from laboratory experiments designed to demonstrate that resistance to endrin in the boll weevil is inherited and to obtain information on the mode of inheritance of resistance. The relative toxicity of endrin to endrin-resistant and -susceptible strains of the boll weevil, F_1 progeny of reciprocal crosses of these strains and F_2 progeny of the crosses were obtained.

METHODS.—A resistant and susceptible strain of the boll weevil of common parental origin were used in the study. The resistant strain was developed by repeated selection with endrin in the laboratory (Brazzel & Lindquist 1960). All boll weevils were reared in the laboratory in petri plates on an artificial diet as described by Brazzel *et al.* (1959).

Reciprocal crosses of the resistant and susceptible strains were made. Approximately 25 males and 25 females that had emerged from the pupal cell during the preceding 6 to 12 hours were placed in each of two 6″×6″×6″ cages, one cage for each reciprocal cross. From 10 to 15 undamaged cotton squares were furnished daily for food and oviposition.

The first- and second-generation progeny of the cross of resistant males by susceptible females were designated strain 1. Progeny of the susceptible male and resistant female were designated strain 2. The F_1 and F_2 generations of the two strains were maintained separately throughout the experiment. The F_1 generation of each

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Table 1.—Toxicity of endrin to resistant and susceptible strains and F_1 and F_2 progeny of reciprocal crosses of the boll weevil.

Strain	No. Weevils	Observation	LD-50 (mg./gm.)	Fiducial Limits .05 Level	
				Upper	Lower
Susceptible (S)	360	24	0.0281	0.0390	0.0216
		48	.0158	.0244	.0111
		72	.0149	.0225	.0095
Resistant (R)	640	24	2.3200	2.4510	2.2910
		48	.8869	.9283	.7177
		72	.2760	.3810	.1810
Cross 1 F_1		24	0.1650	0.2657	0.1206
S Female×	235	48	.1437	.2914	.0945
R Male		72	.1342	.2371	.0903
Cross 2 F_1		24	0.1215	0.1725	0.0931
R Female×	660	48	.0799	.1194	.0562
S Male		72	.0695	.0993	.0489
Cross 1 F_2		24	0.2175	0.2970	0.1620
S Female×	1,050	48	.1183	.1505	.0928
R Male		72	.0890	.1128	.0697
Cross 2 F_2		24	0.3087	0.4088	0.2382
R Female×	1,275	48	.1689	.2126	.1351
S Male		72	.1171	.1470	.0923

strain was allowed to mate *inter se* to produce the F_2 generation. The classes and numbers of boll weevils tested in each class are shown in table 1.

Dosage-mortality curves were obtained for each class of boll weevil by applying scalar dosages of endrin in acetone topically to the metathoracic region of each weevil. Adult weevils that emerged from the larval diet were collected at 2- to 3-day intervals and fed cotton seedlings until 100 weevils had been collected. All weevils were fed at least 2 days before testing. Most of the tests were conducted with weevils 4 to 9 days of age. In a few of the tests weevils as old as 14 days were included. Each test consisted of 5 to 7 dosages plus an acetone-treated check. Groups of 10 to 20 weevils were treated at each dosage and replicated 5 to 18 times.

The weevils were anesthetized with CO_2 and weighed in groups of 10. One microliter of endrin solution was applied to each weevil and allowed to dry before the weevil was released. Treated weevils were held in groups of 10 in half-pint jelly glasses with perforated lids. Cotton seedlings were furnished the treated weevils for food. The insects were held at 83° to 85° F. before and after treatment.

Mortality records were made 24, 48 and 72 hours after treatment. Five levels of response were recorded as follows: (1) apparently unaffected, (2) obviously affected but not down, (3) down and active, (4) down and inactive, and (5) dead. This classification was made in an effort to determine if there was a knockdown and recovery as in the case of house flies (Harrison 1951) and other useful information. For purposes of analysis classifications 1, 2 and 3 were consolidated to form the "live" category and 4 and 5 formed "dead."

Since classification of live or dead is difficult to make as boll weevils commonly feign death, a method for stimulating them was devised. A gooseneck lamp with a 100-watt bulb was placed 5 to 6 inches above a bare table top and allowed to heat the surface upon which the insects were to be placed. The heat was sufficient to elicit an immediate response but not severe enough to harm the weevils.

In order to test the accuracy of the classifications, recounts were made on 20 replications chosen at random and compared with the original count. Only five of the 200 observations were not in agreement and only two of these altered the grosser classifications of "live" and "dead." This indicated that error in classification, a common source of variation in experiments of this sort (Milani 1957), was one of the lesser sources of variation in this experiment.

The average weight of the weevils in each replication was used to determine the effective dosage of insecticide applied and is expressed in milligrams of endrin per gram of weevil weight. Dosage-mortality curves and LD-50's were computed by probit analysis.

Results and Discussion.—The dosage-mortality lines indicating the toxicity of endrin to resistant and susceptible strains, and to F_1 and F_2 progeny from reciprocal crosses of these two strains 24, 48 and 72 hours after treatment, are shown in figures 1, 2 and 3, respectively. The LD-50's for the strains and crosses with fiducial limits are given in table 1.

The regression lines for the F_1 and F_2 progeny of the reciprocal crosses are intermediate between those for the resistant and susceptible parents. Also, the relationship between the six regression lines is essentially the same at 24, 48 and 72 hours after treatment. These results indicate that the resistance is inherited and is not caused by a monogenic dominant or recessive factor, as this would cause a definite shift in the LD-50's of the F_1 and F_2 lines toward one of the parent strains, the direction depending on whether resistance was dominant or recessive. Dominance in a polygenic system would also cause the F_1 and F_2 LD-50's to shift. The intermediate position of the LD-50's for both first- and second-generation progeny indicates no marked dominance of resistance or susceptibility regardless of the number of genes involved. This suggests that resistance in this case is caused largely by additive gene action.

It is not a simple matter to determine if there is only one pair of genes involved or more than one, if resistance is additive. There is no clear-cut evidence in the data presented here to indicate what the situation is with respect to gene number.

Comparison of the LD-50's for the parent strains shows an increase in tolerance to endrin in the resistant strain of 83-fold at 24 hours, 56-fold at 48 hours and 18-fold at 72 hours over the susceptible strain. The LD-50's for the F_1 and F_2 progeny are only slightly different and fall approximately equidistant between the parents. The slope of the regression lines for the parents are steeper than for the progeny of the crosses indicating less variability except for the F_1 progeny from the cross of S female × R male. In this case relatively few weevils were used to obtain the regression line and greater variation was evident in the data. The regression lines for the other F_1

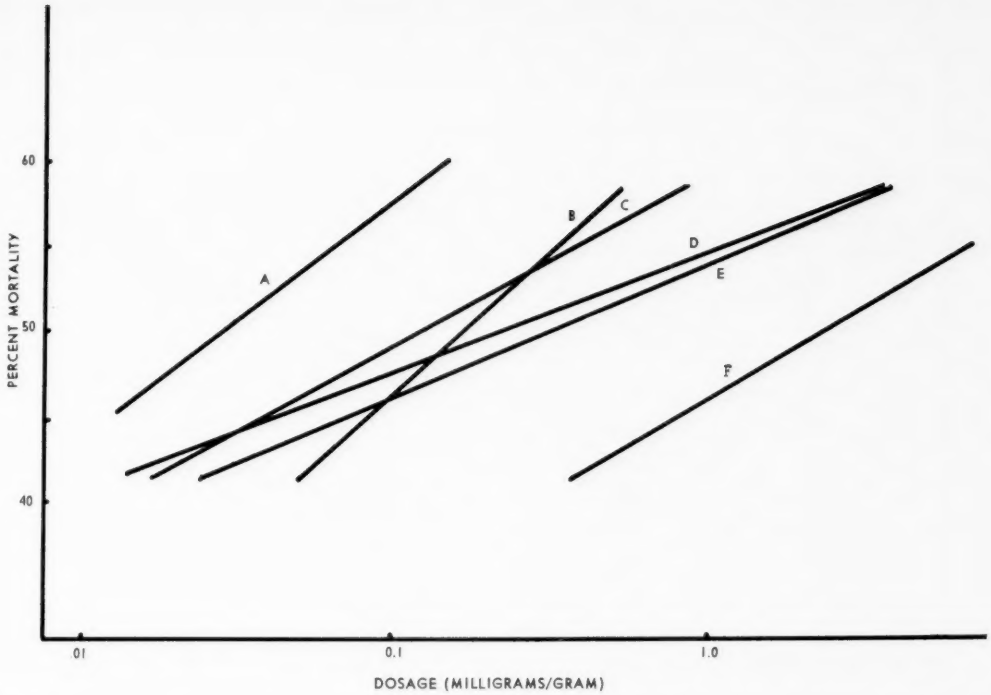

FIG. 1.—Dosage-mortality curves for susceptible (A), resistant (F), F_1 (B) and F_2 (D) of resistant male×susceptible female and F_1 (C) and F_2 (E) of susceptible male×resistant female boll weevils 24 hours after treatment with endrin.

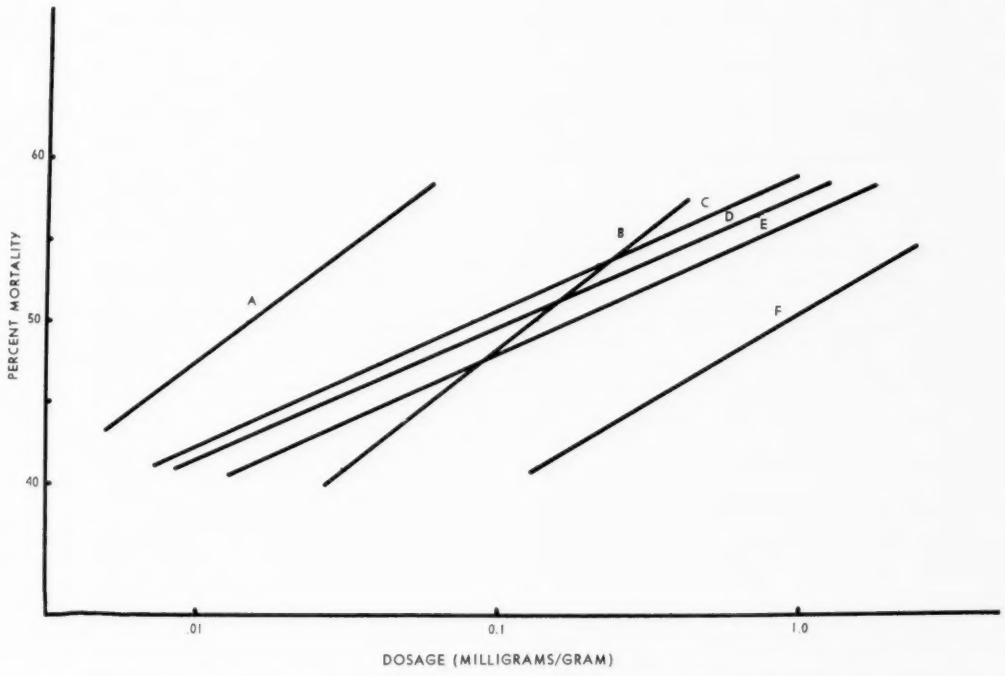

FIG. 2.—Dosage-mortality curves for susceptible (A), resistant (F), F_1 (B) and F_2 (D) of resistant male×susceptible female and F_1 (C) and F_2 (E) of susceptible male×resistant female boll weevils 48 hours after treatment with endrin.

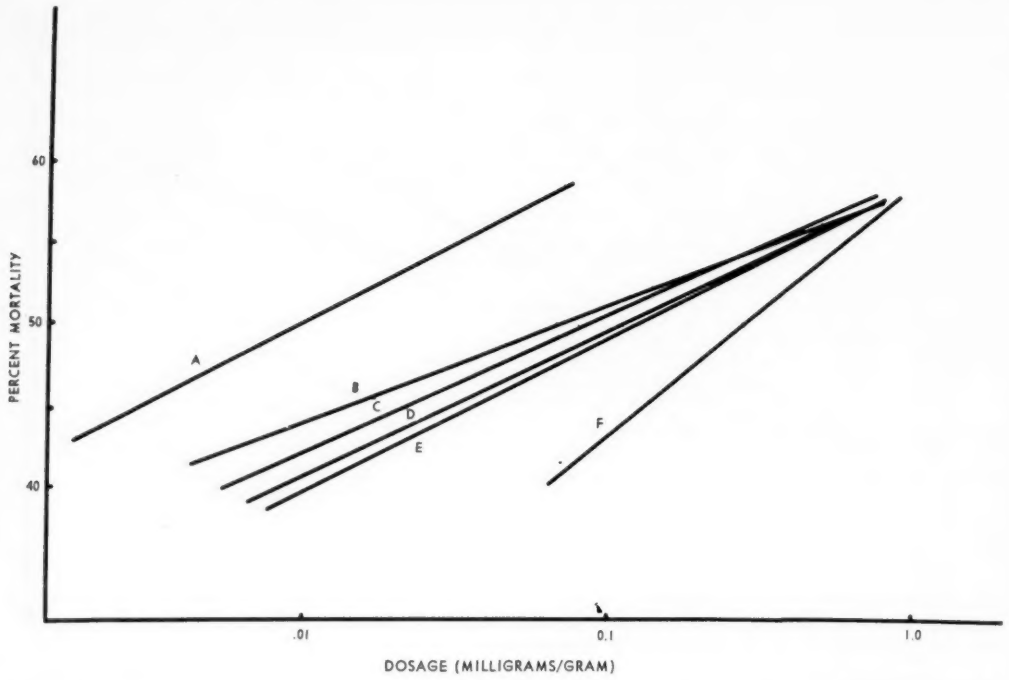

FIG. 3.—Dosage-mortality curves for susceptible (A), resistant (F), F_1 (E) and F_2 (C) of resistant male×susceptible female and F_1 (B) and F_2 (D) of susceptible male×resistant female boll weevils 72 hours after treatment with endrin.

progeny and the F_2 generations are almost parallel, suggesting slight variability due to genetic causes. A wider range of response to dosage in the F_2 compared with the F_1 progeny is the most reliable evidence obtained to support the presence of a polygenic system in these boll weevils. Also, the method of selection used to produce this resistant strain favored this type of genetic system (Milani 1957).

Crosses 1 and 2 represent progeny of different reciprocal crosses made to detect any differences in resistance which might be attributable to sex. The results obtained indicated no evidence of sex linkage of resistance.

Evaluation of the data obtained on knockdown of treated weevils indicated no knockdown and subsequent recovery in the boll weevil as is found in house flies.

The results obtained in this study indicate that resistance to endrin in these strains of the boll weevil is controlled by more than one pair of additive, autosomal genes. However, as pointed out by Crow (1957) a single gene pair with a major effect may be overlooked because of the absence of clear-cut monofactorial ratios, errors in measurements, environmental variability and the all-or-none measurements used. To determine with any degree of certainty if inheritance is monogenic or polygenic, a series of repeated backcrosses to the susceptible strain with mild selection must be undertaken (Busvine 1953).

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Aerial Spray Tests Against Gypsy Moth, *Porthetria dispar*, in New York[1]

D. P. Connola[2] and R. C. Sweet[3]

Abstract

Aerial spray tests against gypsy moth, *Porthetria dispar* (L.), in New York in 1960 showed that the compound Sevin® (1-naphthyl *N*-methylcarbamate) applied at the rate of 1 pound in 1 gallon of spray per acre was as effective as one-half pound of DDT in 1 gallon of spray per acre. In postspray examinations, no egg masses were found in the test plots treated with either material. DDT at one-fourth pound in 1 gallon of spray per acre was not as effective in reducing egg masses. Prespray counts of egg masses in the test plots were from 2,000 to 5,000 per acre. All materials were formulated in No. 2 fuel oil.

Results of airplane spray tests in 1959 showed that the compound Sevin® (1-naphthyl *N*-methylcarbamate) used at the rate of 1¼ pounds per acre was as effective as DDT at one-half pound per acre in controlling gypsy moth, *Porthetria dispar* (L.) in New York (Connola 1960). In the same tests, malathion at 1 pound per acre and Sevin at one-half pound per acre mixed with malathion at one-half pound per acre gave unsatisfactory control of the gypsy moth. All the materials were formulated in fuel oil and applied at the rate of 1 gallon of spray per acre. In the postspray examination, no egg masses were found in the plots treated with 1¼ pounds of Sevin or ½ pound of DDT, whereas prior to spraying, that is, in 1958, the same plots had 700 egg mases and 500 egg masses per acre, respectively.

Methods.—The 1960 tests reported here were based on results of the 1959 tests. In these tests, as in the 1959 tests, woodland areas heavily infested with gypsy moth egg masses were selected for the tests in the fall and winter prior to spraying. Counts of egg masses were made in each plot to serve as a basis for measuring control by the sprays which were to follow after hatching.

As another means of measuring control in the plots, cheesecloth dropping cloths 1 yard by 2 yards in size were erected in hammock-fashion after hatch and just prior to spraying, beneath trees known to be heavily infested with gypsy moth larvae. Ten such dropping cloths were distributed along a transverse string line in each plot. Each cloth was weighted with a heavy stick so that dead larvae and frass droppings from live larvae would collect in the center portion of the cloths and not be easily blown out by wind.

The sprays used in the 1960 tests were formulated in No. 2 fuel oil. In preparing the sprays, the test materials were mixed with the fuel oil so that each gallon of finished spray contained the proper amounts of materials to be tested. Test materials per gallon of spray included 1 pound of Sevin, 1 pound of Sevin plus 4 ounces of Ortho spray sticker, ¼ pound of DDT and ½ pound of DDT. The formulations of Sevin were prepared from "85 P" powder (85% Sevin) especially ground for air application in oil. The Ortho spray sticker is sold commercially and is a resinous type of sticker miscible in either oil or water sprays. The DDT sprays were prepared from a concentrate made by dissolving technical DDT in Amsco HCS solvent (Amsco-Solv H-CS (E-1), American Mineral Spirits Co.). All sprays were applied at the rate of 1 gallon per acre. One-half pound of DDT in 1 gallon of spray per acre is the standard dosage used in gypsy moth control by the New York State Conservation Department.

The sprays were applied on June 8, 1960 by a Stearman airplane with spraying equipment calibrated to deliver 1 gallon of spray per acre with a droplet size of about 175 microns minimum median diameter. Weather conditions at the time of spraying were satisfactory for airplane application. Spray cards impregnated with an oil-soluble dye were used to record the spray deposits in the plots. One card was placed on the ground beneath an opening in the forest canopy near each of the 10 dropping cloths in each plot. The spray deposits recorded on the cards indicated that each of the plots received satisfactory coverage. Larvae at the time of spraying were in the third or fourth instars.

Counts of dead larvae and measurements of frass droppings in the dropping cloths were made just prior to spraying and again at intervals after spraying. The dropping cloths were cleaned of all larvae and frass after each measurement. The frass was measured by the level teaspoonful.

Another means of measuring control by the sprays was the use of a 10-minute count of live larvae. In employing this method, live larvae on the trunks, lower branches and leaves of trees were counted for 10 minutes while slowly following the string line along which the dropping cloths were located. The line not only permitted the larval counts to be made in the same locations each time but also facilitated finding the dropping cloths. The line was attached to the branches of trees and shrubs at about eye level.

Results.—The data given in table 1 show that no egg

Table 1.—Effect of aerial insecticide treatments on abundance of egg masses of the gypsy moth.

Plot No.	No. of Acres Treated	Treatment per Acre	Egg Masses per Acre[a]	
			Prespray (1959)	Postspray (1960)
1	130	1 lb. Sevin + 4 oz. Ortho spray sticker	5,000	0
2	170	1 lb. Sevin	2,000	0
3	100	0.25 lb. DDT + Amsco HCS solvent	2,500	10
4	65	0.5 lb. DDT + Amsco HCS solvent	3,000	0
5	200	Untreated check	2,000	2,150

[a] Prespray figures are estimates based on counts in treated areas made by sampling methods now in use. Postspray figures are actual counts made by thorough examination of $\frac{1}{15}$-acre sample plots in treated areas.

[1] Published by permission of the Director, New York State Museum and Science Service, Journal Series No. 44. Accepted for publication November 14, 1960.

[2] New York State Museum and Science Service, Albany, N. Y.

[3] Bureau of Forest Pest Control, New York State Conservation Department, Albany, N. Y.

Table 2.—Effect of aerial insecticide treatments against the gypsy moth as determined by 10-minute counts of living larvae.

Plot No.	Treatment per Acre	10-Minute Larval Counts			
		Prespray	Postspray		
			June		
		June 7	15	23	30[a]
1	1 lb. Sevin+4 oz. Ortho spray sticker	338	8	8	0
2	1 lb. Sevin	191	36	12	6
3	0.25 lb. DDT+Amsco HCS solvent	244	32	34	8
4	0.5 lb. DDT+Amsco HCS solvent	315	27	1	1
5	Untreated check	180	171	151	84

[a] On June 30 pupation had begun in the plots and the numbers of larvae began to diminish.

masses were found in the plots sprayed with 1 pound of Sevin (with or without sticker) or with one-half pound of DDT per acre. One-fourth pound of DDT per acre was not as effective, having 10 egg masses per acre after treatment. The population of egg masses in the untreated check area remained at about the same level throughout the period of the tests. Postspray counts in all plots were made in late September 1960. At that time $\frac{1}{10}$-acre sample plots in the test areas were examined carefully for egg masses.

Data presented in table 2 show the relative effectiveness of the treatments in reducing the live larval populations in the treated areas. The live larval populations in plot 1 (1 lb. Sevin+sticker) and plot 4 ($\frac{1}{2}$ lb. DDT), which prior to spraying were of about the same magnitude, were comparably reduced after spraying. Also, of the two plots treated with Sevin, there appeared to be a better initial reduction in the plot that was treated with 1 pound of Sevin plus sticker (plot 1) than in the one treated with 1 pound of Sevin alone (plot 2).

Data in table 3 show that, although many larvae were killed in each plot by the spray treatments, there were many live larvae remaining in the plot treated with one-quarter pound of DDT per acre (plot 3) as shown by the continuing production of frass at a high level until pupation about the end of June.

Conclusion.—Although the data on living and dead larvae and frass production as presented in tables 2 and 3 offer a good preliminary index to the relative effectiveness of the treatments, it is the comparison of prespray and postspray counts of egg masses which constitute the best means of measuring control. The egg mass data in table 1 clearly indicate that Sevin applied by airplane in No. 2 fuel oil at the rate of 1 pound in 1 gallon of spray per acre is as effective in controlling gypsy moth as DDT at one-half pound in 1 gallon of spray per acre.

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Table 3.—Effect of aerial insecticide treatments against the gypsy moth as shown by dead larvae and frass on dropping cloths.[a]

Plot No.	Treatment per Acre	No. Dead Larvae[b]					No. Teaspoonfuls of Frass[c]						
		June					Prespray	Postspray					
								June					
		10	13	16	23	30	June 8	10	13	16	23	30	Total
1	1 lb. Sevin+4 oz. Ortho spray sticker	2,132	372	98	21	0	30.0	6.3	2.0	0.8	1.6	0.3	11.0
2	1 lb. Sevin	409	81	35	19	1	19.3	5.9	2.2	0.9	2.1	2.0	13.1
3	0.25 lb. DDT+Amsco HCS solvent	333	133	20	5	4	32.5	14.0	34.5	39.0	20.2	9.8	117.5
4	0.5 lb. DDT+Amsco HCS solvent	191	31	5	5	0	21.5	18.0	7.8	1.5	0.2	0.1	27.6
5	Untreated check	—	0	8	2	0[d]	—	—	12.7	14.1	14.6	9.0[d]	50.4

[a] Numbers of dead larvae and frass measurements are totals from 10 dropping cloths in each plot.
[b] Postspray counts; none found on June 8 before spraying.
[c] Amounts of less than one teaspoonful were estimated.
[d] Seven of the ten dropping cloths were blown clean by a violent storm prior to the frass measurements.

Lysine and Alanine as Mosquito Attractants[1]

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Abstract

L-lysine was found to be an attractant for *Aedes aegypti* (L.) that is present in human blood. L-alanine was also attractive, while L-arginine was slightly attractive. All the other amino acids tested were either not attractive or actually repellent. Nitrogenous bases were either unattractive, *e.g.*, asparagine, ornithine and ammonia, or repellent, *e.g.*, cadaverine and putrescine. Column chromatography showed that the compounds responsible for the attractiveness of 11 different protein hydrolysates, beef blood plasma and human blood plasma were firstly L-lysine, secondly L-alanine, and possibly L-arginine. D-lysine was also attractive. L-lysine was attractive in extreme dilution, *i.e.*, down to 1 p.p.m., and its vapour activated resting mosquitoes. It was attractive also to *Aedes stimulans* (Walker), *Culex pipiens* L., and four strains of *A. aegypti*. Chemical confirmation has been obtained that L-lysine actually distils with water vapour.

The search for compounds in the emanations of vertebrate animals which could be responsible for their attractiveness to mosquitoes has been a long and arduous one. Rudolfs (1922) tested a very large number of substances for attractiveness to *Aedes sollicitans* and *Ae. cantator*, and obtained positive results for benzoic acid, haemoglobin, ammonia, alanine, phenylalanine, aspartic acid, cystine, peptone and bouillon. Reuter (1936) tested the acids and bases mentioned above on *Anopheles atroparvus* and found them unattractive along with other sweat acids, indole, skatole and trimethylamine. DeLong (1949) reported that sweat substances such as lactic acid, methyl caprate, sodium acrylate and tributyrin did not increase the attractiveness of a warm moist object to *Ae. aegypti*. Brown *et al.* (1951) found that acetic acid, lactic acid, ammonia, trimethylamine and ethyl butyrate showed no consistent attractiveness to *Ae. aegypti*.

The search was stimulated by the report of Schaerffenberg & Kupka (1951) that they had obtained a blood distillate which was attractive to *Culex pipiens* down to a dilution of 2000 times in water. Burgess & Brown (1957) then found that CO_2 was responsible for only part of the attractiveness of beef blood plasma to *Aedes aegypti*. Recently, Schaerffenberg & Kupka (1959) reported that a mixture of alanine, cystine, cysteine, glutamic acid, lactic acid, methylamine, dimethylamine, trimethylamine and ammonia was attractive to *C. pipiens* and *An. atroparvus* at concentrations down to 0.05%; however, none of these compounds proved attractive when tested singly.

The studies reported below commenced with the report of Rudolfs (1922) that peptone was attractive, and terminated with the discovery that lysine and alanine are highly attractive blood constituents for *Ae. aegypti*, lysine being still active at 1 p.p.m. dilution in water.

Biological Material and Methods.—The mosquitoes used in the tests were adult females of the Guelph strain of *Aedes aegypti* (L.), which originated from a single female of the well-known Orlando laboratory strain. The larvae were reared in distilled water containing 15 p.p.m. streptomycin, and were fed every 2 days on a mixture of 1 part blood albumin to 10 parts brewers' yeast powder.

The cage in which the emerged adults were used for test was designed by Burgess & Brown (1957); made of wire screening, it measured 35×35×24 inches and offered the mosquitoes enough room for free flight (fig. 1). It was charged with 3 to 4 thousand pupae, and the mosquitoes were used between 2 and 27 days after emergence. During this period they were not given blood meals, but had free access to 10% sucrose absorbed on cotton-gauze pads, and to water-soaked raisins suspended on cotton thread. For subsequent testing, the cage was cleared and a new lot of pupae introduced. The males did not enter into the picture, since they seldom responded in the tests and if so could be immediately recognized, and they died out within a few days.

The compounds to be tested were dissolved in water, and 5-ml. aliquots of the test solution and control liquid were added to pads consisting of three discs of No. 1 Whatman filter-paper fitted into 9-cm. petri dishes. These were warmed to 99° F. on an aluminum tray over a hotplate, and were set in each of two holes 12 inches apart recessed into the top of a black box (27×8×7 inches) on the bottom of the free-flight cage (fig. 1). The dishes thus exposed each rested on a closed 16-oz. metal-topped glass jar filled with water at 150° F. The mosquitoes were observed through a glass window in the front of the cage; a deflection board placed above this window further prevented the observer's breath from entering the cage. In one experiment, yet further precautions to minimize breath and body odours were taken by enclosing the observer in an airtight plastic suit (Burgess 1959), from which the expired CO_2 was pumped outside the building.

The numbers of approaches made by the mosquitoes within 0.5 inches of either petri dish were counted with the aid of a backing of white index-card, 0.5 inches high, installed behind each dish. A complete test consisted of eight paired counts of 0.5-minute duration at 1-minute intervals. The approaches to either dish were counted simultaneously by a single observer with the aid of hand-tally counters held in each hand. After every count the dishes were interchanged, being re-warmed in the process and always handled with bottle forceps. The results were expressed as the "Attractiveness Ratio," *i.e.*, the number of approaches to the experimental solution divided by that to the control liquid. The variance was such that a significant difference at the 5% level of probability was generally shown by tests giving attractiveness ratios above 1.24. The environmental conditions during the tests were 80° F. and 75% relative humidity.

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[2] The authors are indebted to L. Burgess, Entomology Laboratory, Canada, Department of Agriculture, Guelph, Ont. for the mosquito strain employed, for recommending the larval medium, for collecting wild-caught mosquitoes, and for lending the airtight plastic suit.

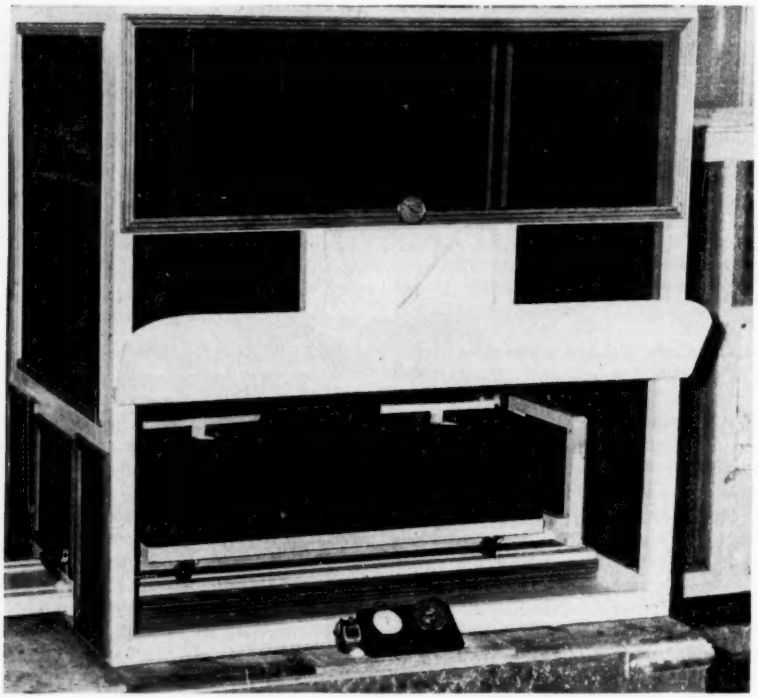

FIG. 1.—Test cage, showing two petri dishes recessed in holes in the black box.

BIOCHEMICAL MATERIAL AND METHODS.—Many of the protein hydrolysates tested were obtained from Difco Laboratories, Detroit, Michigan. An equal number of hydrolysates, and all of the amino acids, were obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio. Other hydrolysates were obtained from spray-bait manufacturers. Blood was obtained from cattle at time of slaughter, 1 litre being collected in a sterile cotton-stoppered flask containing 50 mg. of heparin in 16 ml. of 0.9% NaCl, refrigerated and used not less than 5 hours later. Human blood preserved with "acid citrate dextrose" was obtained through the local blood bank from a recent donor, and used 3 hours after being drawn. Blood plasma was obtained by centrifuging twice at 2,000 r.p.m. for 20 minutes.

Distillates of the various protein hydrolysates were obtained at 37° C. and 7 mm. pressure from 100 ml. of a 10% solution placed in a vertical 250-ml. side-arm flask, connected to a similar flask whose side-arm led to a vacuum pump and manometer. The first flask was kept in warm water at 37° C., and the second or receiver flask was immersed in a mixture of ice and acetone. Continuous operation of the vacuum pump reduced the pressure to a steady 7 mm. Hg., and the solution distilled without bumping or droplet formation; distillation was maintained for 7 to 12 hours until the solution had gone to dryness. The residue was dissolved in 100 ml. water, and this aqueous residue and the aqueous distillate were tested for attractiveness. Distillates of blood plasma and of a 0.7% solution of lysine were obtained similarly.

For separation by column chromatography, lactalbumin hydrolysate was first deproteinized with 4N HCl, using 2 ml. and 5 ml., respectively, for 100 ml. of the 0.1% and 10% solutions of the hydrolysate; a 10-ml. aliquot was then centrifuged at 3,500 r.p.m. Blood plasma was deproteinized with 1.0% picric acid, using 500 ml. per 100 ml. plasma in a glass-stoppered flask (Stein & Moore 1954). After shaking, the suspension was centrifuged for 10 minutes, and the supernatant was passed through a bed of Dowex 2-X8 resin to remove the picric acid. The deproteinized plasma solution was concentrated to a volume of 2 ml. by distillation at 37° C. and 7 mm. Hg.

Column chromatography was performed at pH 5.0 with the styrene divinylbenzene resin Dowex 50W-X4 (200-400 mesh, obtained from J. T. Baker Chemical Co., Phillipsburg, N.J.) prepared as its sodium salt by washing with HCl and then NaOH (Moore & Stein 1951). The salt was again washed in 1N NaOH, suspended in 0.2N sodium citrate-acetate buffer at pH 5.0, and poured in five successive lots to give a 50×1.2 cm. column (Moore & Stein 1954). A 250-ml. separatory funnel was attached to the top of the column, and at least 100 ml. of 0.2N NaOH was passed through it overnight. This was followed by 200 ml. of 0.2N sodium citrate buffer at pH 3.1, and when the pH of the eluate had reached pH 3.1 the column was ready for chromatography.

The solutions to be chromatographed were adjusted to pH 2.2 with 4N HCl, and aliquots of 1 to 3 ml. (exact amount stated in results) were added to the surface of the

column with a bent pipette. The sample was followed with 1 ml. of sodium citrate buffer at pH 2.2 and then 2 ml. of buffer at pH 3.1. Then the chromatography tube and separatory funnel were filled with sodium citrate-acetate buffer at pH 5.0 and elution was commenced. The column delivered eluate at a rate approximating 4 ml. per hour, and successive 1-hourly samples were collected by an automatic fraction collector. The column was operated at 76° F. in a constant-temperature room.

The separate fractions were tested for their attractiveness to the mosquitoes after making them alkaline (pH 10) with a few drops of 4N NaOH, being compared with the citrate-acetate buffer similarly adjusted to pH 10. Small aliquots (0.5 ml.) were also tested for the presence of amino acid by the ninhydrin method (Moore & Stein 1948). The first ninhydrin-positive fraction appeared at 8 to 9 hours (32–39 ml. eluate) after the addition of the sample to the column, and the eluate remained positive for 31 to 32 hours (124–128 ml. eluate), when the fractionation was terminated. Each hourly fraction, after being made alkaline and tested with ninhydrin, was approximately 3.8 ml. in volume and was tested for attractiveness to mosquitoes against 3.8 ml. of the citrate-acetate buffer adjusted to pH 10.

The presence of lysine or alanine in those eluate fractions which showed attractiveness was established by paper chromatography. The buffer salts were first removed from an aliquot of the fraction by passing it through acidified Dowex 50W-X4 resin according to the method of Dreze & De Boeck (1952). Then the samples, after concentration seven times, were compared with known amino acids by descending paper chromatography in a water-phenol system (Linstead *et al.* 1955), the spots being developed with 0.1% ninhydrin in water-saturated n-butanol.

RESULTS.—Initial studies investigated the possible attractiveness of peptones and a great variety of protein hydrolysates. Positive results being obtained, assessments were then made of the individual amino acids present in the most attractive of the hydrolysates. Lysine and alanine proving strong attractants, the final studies involved the chromatographic fractionation of lactalbumin hydrolysate, beef blood and human blood, and associating the attractive fractions with lysine and alanine.

Protein hydrolysates.—Of the 16 different protein hydrolysates tested as 10% solutions, 11 proved to be significantly attractive (table 1), and eight of them were more attractive than beef blood plasma. When seven of the hydrolysates were distilled at reduced pressure, it was found that five of the distillates were attractive (table 2), while all seven of the nondistilled residues remained attractive. The distillate from lactalbumin hydrolysate gave the highest attractiveness ratio, it being 3.03 in comparison with water. On dilution of this distillate by 3, 9 and 27 times with water, the attractiveness ratios fell to 1.57, 1.34 and 1.10, respectively.

Table 1.—Attractiveness to female *Aedes aegypti* of 16 protein hydrolysates as compared with blood plasma.

HYDROLYSATES (10% SOLUTIONS)	NUMBER OF MOSQUITO APPROACHES		ATTRACTIVENESS RATIO
	Water	Hydrolysate	
Blood plasma, beef	422	623	1.48
Peptonized milk hydrolysate[a]	434	1,494	3.44
Brewers yeast autolysate[c]	489	1,589	3.25
Casein hydrolysate, enzymatic[b]	353	962	2.72
Yeast hydrolysate, enzymatic[b]	313	572	1.83
Protein digest proteoses (Bacto-Protone)[a]	408	742	1.82
Casein digest, pancreatic (Bacto-Casitone)[a]	330	598	1.80
Soy hydrolysate, enzymatic[b]	259	447	1.72
Lactalbumin hydrolysate, enzymatic[b]	631	957	1.51
Casein hydrolysate, acid[b]	358	527	1.47
Gelatin hydrolysate[a]	721	1,018	1.41
Protein digest, enzymatic (Neopeptone)[a]	213	207	0.97
Proteose-Peptone No. 2[a]	260	225	0.86
Blood hydrolysate, enzymatic[b]	490	340	0.71
Protein insecticide bait No. 2[d]	204	121	0.59
Protex hydrolysed protein[e]	342	176	0.52

[a] Difco Laboratories, Detroit 1, Mich.
[b] Nutritional Biochemicals Corporation, 21010 Miles Ave., Cleveland 28, Ohio.
[c] Amber Laboratories, 3456 North Buffum St., Milwaukee 12, Wis.
[d] A. E. Staley Co., Decatur, Ill.
[e] Fredk. Boehm Ltd., 19 Bentinck Street, London W. 1., England.

Amino Acids.—The natural L-isomers of 17 amino acids were individually tested for attractiveness in the concentration at which they would be expected to occur in the 10% lactalbumin hydrolysate (Mitchell 1946). The results, involving four to eight complete tests with each in comparison with distilled water (table 3), indicate that most of the amino acids are inactive, certain of them even tending to be repellent. Arginine showed an attractiveness ratio of 1.24, difference from unity being just below the borderline of significance at the 5% level; alanine showed somewhat greater attractiveness (ratio 1.32). The amino acid that was strongly attractive was L-lysine (free-base), with an attractiveness ratio of 3.83.

A mixture of all the amino acids except lysine was weakly attractive; addition of lysine made the mixture highly attractive (table 4). It also raised the pH of the mixture from 3.4 up to 4.4. However, the results were similar when the mixture with or without lysine was tested

Table 2.—Attractiveness to female *Aedes aegypti* of seven protein hydrolysates before and after distillation.

HYDROLYSATE	UNDISTILLED		DISTILLATE			RESIDUE		
	pH	Attr. Ratio	Control	Expt'l.	Attr. Ratio	Control	Expt'l.	Attr. Ratio
Blood plasma, beef	7.4	1.48	434	731	1.68	221	347	1.57
Peptonized milk hydrolysate	6.2	3.44	757	1,379	1.82	401	839	2.09
Casein hydrolysate, enzymatic	6.6	2.72	581	1,320	2.27	161	570	3.54
Yeast hydrolysate, enzymatic	5.2	1.83	1,052	245	0.23	383	1,144	2.99
Protein digest proteoses (Bacto-Protone)	7.5	1.82	291	497	1.71	152	230	1.51
Casein digest, pancreatic (Bacto-Casitone)	7.1	1.80	473	465	0.98	291	718	2.47
Soy hydrolysate, enzymatic	7.3	1.72	282	665	2.36	96	151	1.57
Lactalbumin hydrolysate, enzymatic	6.6	1.51	176	528	3.00	165	353	2.14

Table 3.—Attractiveness to female *Aedes aegypti* of individual L-amino-acids at concentrations obtaining in 10% lactalbumin.

Amino Acid	Number of Mosquito Approaches: Water	Number of Mosquito Approaches: Amino Acid	Attractiveness Ratio
Lysine, 0.88%	675	2,586	3.83
Alanine, 0.24%	257	338	1.32
Arginine, 0.30%	131	162	1.24
Tryptophane, 0.27%	154	162	1.05
Phenylalanine, 0.13%	132	129	0.98
Glutamic acid, 1.3%	221	209	0.96
Cystine, 0.11%	364	347	0.95
Serine, 0.18%	291	259	0.89
Methionine, 0.88%	285	250	0.88
Valine, 38%	178	151	0.85
Glycine, 0.04%	228	185	0.81
Histidine, 0.21%	282	210	0.75
Isoleucine, 0.70%	402	303	0.75
Aspartic acid, 0.82%	388	272	0.70
Proline, 0.38%	337	232	0.69
Leucine, 0.70%	150	102	0.68
Tyrosine, 0.05%	437	255	0.58

Table 4.—Attractiveness to female *Aedes aegypti* of amino-acid mixtures with or without L-lysine.

Mixtures	pH	Number of Mosquito Approaches: Water	Number of Mosquito Approaches: Amino-Acid Mixture	Attractiveness Ratio
16 Amino acids without Lysine	3.4	845	961	1.14
16 Amino acids plus Lysine	4.4	695	1,944	2.80
16 Amino acids without Lysine	7.4	279	360	1.28
16 Amino acids plus Lysine	7.4	32	196	6.13

Table 5.—Attractiveness to female *Aedes aegypti* of L-lysine and its hydrochloride at various pH levels.[a]

pH	Buffer	No. of Mosquito Approaches: Control	No. of Mosquito Approaches: Lysine	Attractiveness Ratio
	L-lysine (free-base) 0.70%			
4.40	Phthalate-phosphate	49	276	5.62
7.45	Phthalate-phosphate	248	802	3.23
7.45	Na Citrate-acetate	138	420	3.04
9.52	No buffer	675	2,586	3.84
9.73	Tetraborate-carbonate	212	1,079	5.09
	L-lysine Mono-HCl 0.88%			
5.52	Phthalate-phosphate	285	183	0.64
5.95	No buffer	767	861	1.12
6.73	Tetraborate-phosphate	358	465	1.30
7.38	Tetraborate-phosphate	70	182	2.59
11.20	4N NaOH	121	404	3.34

[a] Control solutions are identical except for absence of amino acids.

at 7.4, the pH of human blood. L-lysine is a basic amino acid, and dissociates in water to give a pH of 9.47. It is however attractive in acid as well as alkaline solution (table 5). On the other hand L-lysine monohydrochloride proved to be unattractive in acid solution, but its attractiveness increased as conditions were made more alkaline.

Distillates from 0.7% lysine solutions proved to be highly attractive (table 6). After being concentrated 200 times at 100° C., the distillate gave a positive test with ninhydrin, the threshold of sensitivity of the test being 100 p.p.m. lysine. It is probable that the lysine distils mainly when the solution approaches dryness, since the distillate was unattractive at first (6 hours) but attractive when dryness was reached at 12 hours.

Table 6.—Attractiveness of the distillate and residue of 0.7% L-lysine to female *Aedes aegypti*.

Expt. No.	Attractant	Number of Mosquito Approaches: H_2O Control	Number of Mosquito Approaches: Experimental	Attractiveness Ratio
1	Distillate	116	897	7.73
	Residue	71	643	9.06
2	Distillate at 6 hrs.	144	140	0.97
	Distillate at 12 hrs.	64	142	2.23
	Residue	56	490	8.75

Successive dilutions of L-lysine in sodium tetraborate-carbonate buffer at pH 10 were tested for attractiveness against the buffer control (table 7). The lysine was attractive down to a concentration of 0.0001% (1 p.p.m.) but not at 0.1 p.p.m. When the observer was enclosed in an airtight plastic suit to exclude breath and body odours, 0.88% lysine was no less attractive. The unnatural isomer D-lysine, obtained and tested as the hydrochloride, resembled L-lysine hydrochloride in being slightly attractive in neutral and highly attractive in alkaline solution; at pH 6.5 a solution of 0.88% D-lysine-HCl gave an attractiveness ratio of 1.20, which increased to 3.70 when the solution was made alkaline to pH 11.5.

L-lysine proved to be attractive also to other strains of *Aedes aegypti* from Key West, Trinidad and Penang (table 8); these strains were in regular culture and had been given daily access to a guinea pig before being tested. L-lysine also proved attractive to adult *Aedes stimulans* (Walker) caught in the field on May 20, 1960 in the vicinity of Guelph, Ont. Positive results could not be obtained with adult *Culex pipiens* L. in an artificially lit rearing-room during the day, but when the illumination was reduced at evening (a regular rearing practice) sufficient adults responded to show the attractiveness of lysine.

Table 7.—Attractiveness to female *Aedes aegypti* of extreme dilutions of L-lysine, and of 0.88% lysine in absence of observer odours.

Attractant	pH	Number of Mosquito Approaches: Control	Number of Mosquito Approaches: L-lysine	Attractiveness Ratio
L-lysine 0.01%	10.0	24	146	6.08
0.001%	10.0	63	374	5.95
0.0001%	10.0	55	157	2.86
0.00001%	10.0	44	31	0.71
L-lysine 0.88%[a]	9.5	46	208	4.30
D-lysine-HCl 0.88%	6.5	218	261	1.20
	11.5	50	185	3.70

[a] Observer enclosed in a plastic suit.

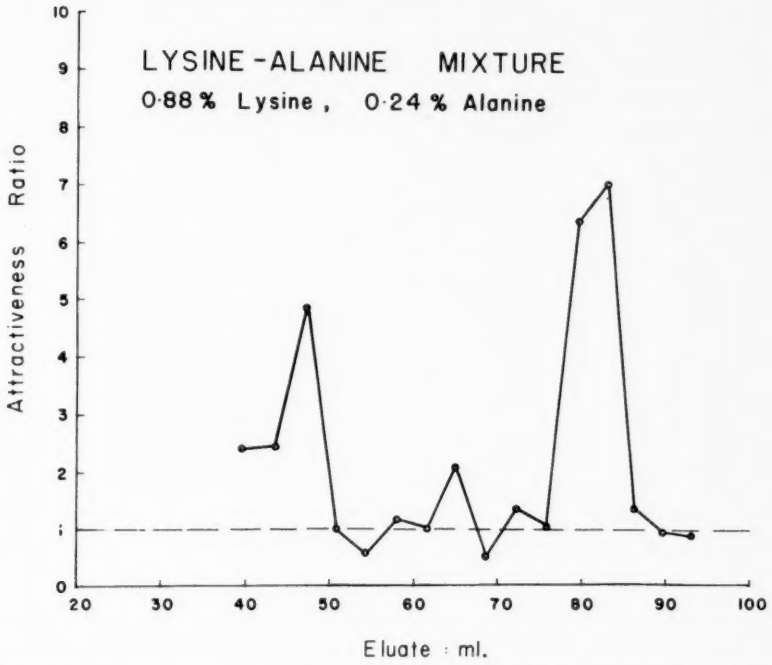

FIG. 2.—Attractiveness of chromatographed fractions of a mixture of 0.24% alanine and 0.88% lysine.

L-alanine had at first proved only weakly attractive, a 0.24% solution at its pH of 8.1 showing an attractiveness ratio of 1.32, as compared with 3.83 for 0.88% L-lysine (table 3). When a mixture of 0.24% alanine and 0.88% lysine was chromatographed (see below, fig. 2), L-alanine which eluted first showed an attractiveness ratio of 4.87, as compared with 7.00 for L-lysine which eluted later (table 9). When individually tested at pH 7.4, a solution of 0.5% alanine compared with its citrate-acetate buffer control showed an attractiveness ratio of 1.82, whereas 0.5% lysine showed a ratio of 2.14. When the two 0.5% solutions were directly compared one with the other, L-alanine proved to be almost as attractive as L-lysine.

Chromatographic Fractionation.—Lactalbumin hydrolysate was compared with lysine-alanine mixture to ascertain whether the peaks of attractiveness in the eluate were congruent. When 2 ml. of a solution containing 0.24% L-alanine and 0.88% L-lysine was chromatographed through Dowex 50W-X4 resin and the eluate fractions assessed by exposure to the mosquitoes (fig. 2), the attractiveness of alanine appeared at 40 to 50 ml. and that of lysine at 75 to 88 ml. after the addition of the sample to the column. When a 1-ml. sample of deproteinized 10% lactalbumin hydrolysate was similarly chromatographed (fig. 3), the eluate fractions showed considerable attractiveness at 66 to 90 ml., with a minor peak at 53 ml. When the chromatography was repeated with 0.1% lactalbumin hydrolysate, the peak of attraction was at 87 ml. This fraction was tested by descending paper chromatography and found to contain L-lysine. Both samples of lactalbumin hydrolysate showed a minor peak of attractiveness at about 118 ml., which may be due to L-arginine.

Beef blood plasma was next chromatographed, a 100-ml. and a 10-ml. sample each being deproteinized and concentrated to 2.4 ml. for addition to the column (fig. 4). Both samples showed the main peak of attractiveness at approximately 90 ml., with smaller peaks at 40 to 50 ml. and slight attractiveness at 110 to 130 ml. The amino acid present in the 93-ml. and 88-ml. fractions of either sample was identified as L-lysine by paper chromatography.

Human blood plasma was assayed as a 75-ml. and a 7.5-ml. sample each deproteinized and concentrated to 3 ml. for chromatography (fig. 4). The eluates of both samples showed peaks at 40 to 50 ml., around 80 ml., and at 110 to 120 ml. From the first sample, the eluate fraction at 44 ml. was found by paper chromatography to contain L-alanine, and the fraction at 81 ml. to contain L-lysine.

Special precautions were taken against being misled by contaminants of these amino acids; for example, ammonia elutes close to lysine (Moore & Stein 1954). Ammonia was found to be repellent, and not to become attractive as the concentration was reduced (table 10). Ornithine elutes

Table 8.—Attractiveness of 0.88% L-lysine to different strains and species of mosquitoes.

SPECIES	STRAIN	NUMBER OF MOSQUITO APPROACHES		ATTRACTIVENESS RATIO
		Water	Lysine	
Aedes aegypti	Key West	42	222	5.29
	Orlando	675	2,586	3.83
	Penang	135	483	3.58
	Trinidad	44	127	2.89
Aedes stimulans	Guelph, Ont.	12	27	2.25
Culex pipiens	Guelph, Ont.	03	13	4.33

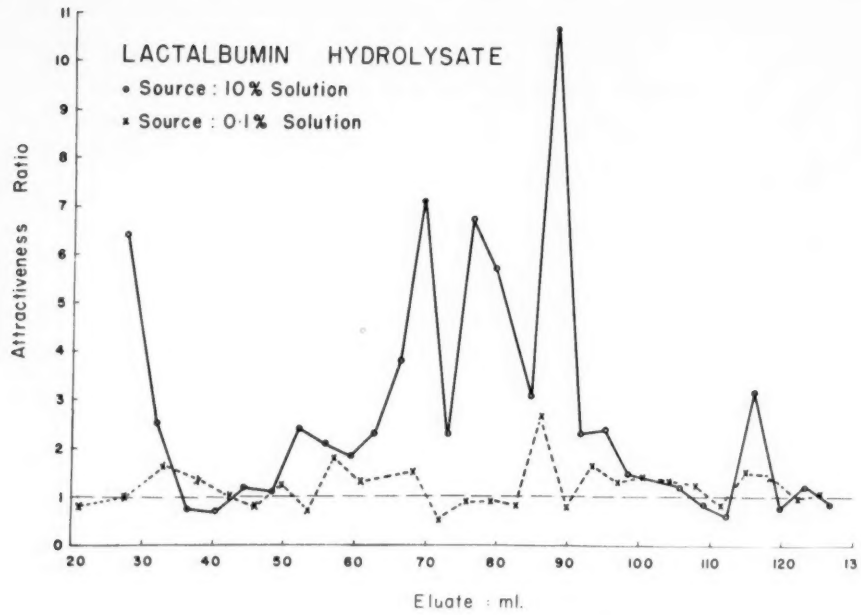

FIG. 3.—Attractiveness of chromatographed fractions of 10% and 0.1% lactalbumin hydrolysate.

even closer to lysine, and asparagine close to alanine; but ornithine mono-HCl and asparagine were found to be unattractive even in alkaline solution. The dibasic amine cadaverine is closely related to lysine and therefore could be suspected as a contaminant; but it proved to be strongly repellent and did not become attractive even when diluted to 0.01 p.p.m. The diamine putrescine was also repellent.

DISCUSSION.—The results obtained with the chromatographic fractionation of blood plasma indicate that lysine is the most attractive component for culicine mosquitoes. The attractiveness of certain protein hydrolysates also appears to be mainly due to lysine. It is unlikely that this finding is an artifact due to contamination, since evidently lysine can volatilize, and the possible contaminants appear to be repellent. When a solution of lysine is exposed in a cage, the resting mosquitoes are stimulated to take flight, to approach and repeatedly sample the source, occasionally landing on it; these responses closely resemble those made to a guinea pig or a human hand introduced into the cage.

Alanine also excites these responses, but not so strongly. It is a weaker attractive component in blood and protein hydrolysates, although showing up strongly in the fractionation of human plasma. It did not render an amino-acid mixture attractive as lysine did. Probably its effect is not strong enough to operate when in admixture with other amino acids, several of which appear to be actually repellent. In biological fluids, further competition would be offered from the nitrogenous bases shown above to be repellent. Alanine was one of the amino acids reported as attractive by Rudolfs (1922) and as a component of the attractive mixture of Schaerffenberg & Kupka (1959), whereas lysine has not been previously investigated.

Lysine and alanine are two of the three most abundant amino acids in human blood (Stein & Moore, 1954). Lysine is also present in sweat (Hier *et al.* 1946) but this secretion contains a great number of acids and bases many of which are probably repellent. Thus many workers (Howlett 1910, Crumb 1922, Rudolfs 1922, Reuter 1936) have found no attractiveness in sweat for *Aedes*, *Culex* or *Anopheles*. Sweat has proved significantly attractive only under certain conditions and in certain concentrations (Parker 1948, Brown 1951, Thompson & Brown 1955).

Body odour, on the other hand, is consistently attrac-

Table 9.—Attractiveness to female *Aedes aegypti* of L-alanine in comparison with L-lysine.[a]

ATTRACTANT	L-ALANINE			L-LYSINE		
	Control	Expt'l.	Attr. Ratio	Control	Expt'l.	Attr. Ratio
Concentration in table 3	257	338	1.32	675	2,586	3.83
Eluate from mixture	96	467	4.87	14	98	7.00
0.5% solutions at pH 7.4	353	641	1.82	128	274	2.14
L-alanine vs. L-lysine	—	229	—	—	243	1.06

[a] For explanation see text.

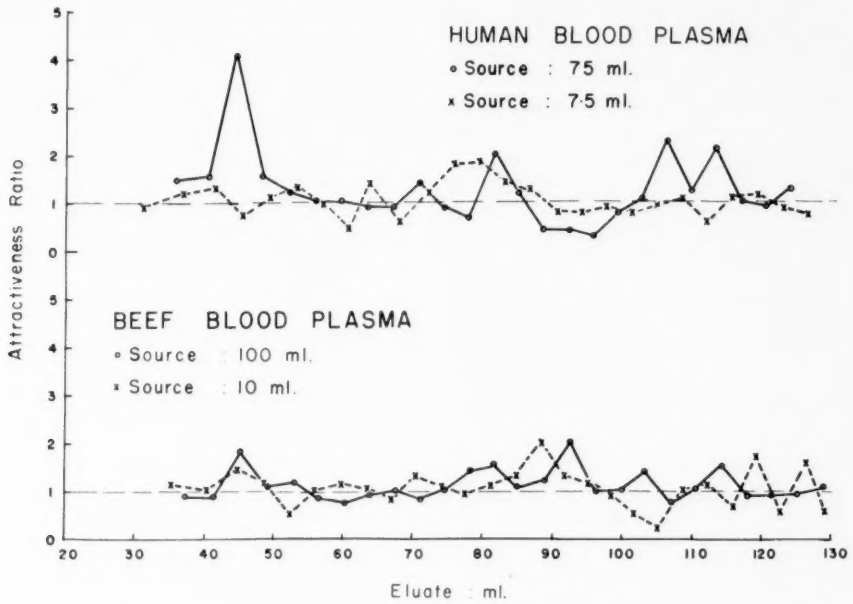

FIG. 4.—Attractiveness of chromatographed fractions of 100-ml. and 10-ml. samples of beef blood, and of 7·5-ml. and 7·5-ml. samples of human blood.

tive, and Willis (1947) has demonstrated the vapour effect of the human arm on *Aedes* and *Anopheles* in an olfactometer. Laarman (1955) further demonstrated that the attractiveness of a vapour stream from a rabbit to *An. atroparvus* was only partially reduced when the CO_2 was removed. Rahm (1957) found with *Ae. aegypti* that although sweat from the hand was significantly attractive, the odour volatilized from the hand was so much more attractive that sweat normally did not matter.

It is the opinion of Schaerffenberg & Kupka (1959) that the source of body odour is the blood itself. Although Howlett (1910) and Rudolfs (1922) found blood to be unattractive to *Aedes* mosquitoes, it has been found attractive to *Anopheles* by van Thiel & Weurman (1947) and Laarman (1955), and to *Aedes aegypti* by Burgess & Brown (1957) and the present workers. That mosquito attractants can diffuse from the skin capillaries has been suggested by Schaerffenberg & Kupka (1959), and their volatilization with water vapour has been demonstrated in the present study.

Previous work has made it clear that attractiveness is due to a number of factors—warmth, moisture, CO_2, body odour and visual stimuli—and that their relative importance varies according to the situation (Brown 1956). It is likely that the principal factor in preferential host selection, which differs from one species of *Anopheles* to another (van Thiel 1939), is that of body odour. Thus it becomes possible that the basis of host preferences of various species of mosquitoes, and even of anthropophilic and zoophilic strains of the same species, may be sought in particular amino acids in the body emanations, of which alanine is one but the most important is lysine.

Table 10.—Attractiveness to female *Aedes aegypti* of aliphatic amines, ornithine and ammonia.

ATTRACTANT	pH	NUMBER OF MOSQUITO APPROACHES: H_2O Control	NUMBER OF MOSQUITO APPROACHES: Experimental	ATTRACTIVENESS RATIO
Ammonia, 0.0005%		255	240	0.94
0.002%		332	227	0.68
0.01%		286	229	0.80
0.04%		138	82	0.59
0.20%		126	63	0.50
L-Ornithine Mono-HCl, 0.88%	11.60	54	55	1.02
L-Asparagine, 0.5%	11.22	181	222	1.22
Putrescine Di-HCl 0.88%	7.05	67	46	0.69
0.88%	11.08	108	15	0.14
Cadaverine Di-HCl 0.88%	7.20	245	121	0.49
0.88%	11.08	94	7	0.08
0.01%	10.0	227	72	0.32
0.001%	10.0	202	113	0.56
0.0001%	10.0	153	87	0.57
0.00001%	10.0	120	107	0.89
0.000001%	10.0	82	78	0.95

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Notes on Miscellaneous Insects of Arizona[1]

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ABSTRACT

There are some 270 Arizona species of insects listed in this paper which were collected by the writer and his associates from 1953 to 1959, mostly incidental to economic insect control. However, some of the records were acquired by exploratory collecting. Notes immediately following each species in the list give the host plant(s), host insect(s) or habitat(s), place(s) and date(s) of collection, collector(s) if other than the writer or if in partnership with him, and with some a notation as to degree of infestation and/or other information.

The following orders are represented: Coleoptera, Dermaptera, Diptera, Embioptera, Hemiptera, Hymenoptera, Lepidoptera, Psocoptera, and Thysanoptera. Taxonomists who made the identifications are listed and 15 references are cited.

Below is an annotated list of insects of Arizona taken from records accumulated from 1953 to 1959 based on collections by the writer and his associates and identifications by taxonomic specialists[3] of the various groups. Each species, under its respective order (or suborder) by family (or superfamily), is listed with notes immediately following it, giving its host plant(s), host insect(s) or habitat(s), place(s) and date(s) of collection, collector if other than the writer or if in partnership with him.

The list is based mostly on collections made incidental to economic entomological activities. However, some of the records were obtained solely to become better acquainted with the insects of the area. Many additional records were included in other papers: some of thrips (Bibby 1958), aphids, psyllids and whiteflies (Bibby 1959), and of spider mites (Bibby & Tuttle 1959). Nothing of Orthoptera is included because most of it is well covered in a publication by Ball *et al.* (1942) and the same is applicable to predaceous syrphids by Butler and Werner (1957).

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[2] Now retired, P.O. address, Smithville, Mississippi. The writer is very grateful to M. F. Wharton of the Arizona Fertilizer and Chemical Company, for encouraging him to, and insisting that he, make collections and records of Arizona insects; to P. W. Oman of the U. S. Department of Agriculture for referring innumerable specimens to taxonomists for identification and for transmitting their determinations to him; and to the taxonomists listed below for their many favors.

[3] W. H. Anderson, R. H. Arnett, W. F. Barr, R. S. Beal, Jr., L. J. Bottimer, E. A. Chapin, G. H. Nelson, M. W. Sanderson, R. B. Selander, T. J. Spilman, Barry D. Valentine, Patricia Vaurie, G. B. Vogt, Rose E. Warner, F. G. Werner: Coleoptera. H. B. Mills: Collembola. A. B. Gurney: Dermaptera. R. H. Foote, C. W. Sabrosky, W. W. Wirth: Diptera. E. S. Ross: Embioptera. Jose C. M. Carvalho, H. M. Harris, R. I. Sailer: Heteroptera. T. E. Moore, Harold Morrison, Louise M. Russell, D. A. Young: Homoptera. B. D. Burks, K. V. Krombein, C. F. W. Muesebeck, Luella M. Walkley: Hymenoptera. H. W. Capps, J. F. G. Clarke, E. L. Todd: Lepidoptera. Kathyrn M. Sommerman: Psocoptera. Kellie O'Neill: Thysanoptera.

COLEOPTERA

ANTHRIBIDAE

Trigonorhinus sp.

Franseria deltoidea: Mesa and Tempe, II-17 and 24–54.

Trigonorhinus limbatus (Say)
griseus (LeC.)

Actinea odorata: Pinal Co., IV-3-59.

BRUCHIDAE

Acanthoscelides griseolus (Fall)

Lettuce: Yuma, XII-1-53.

Algarobius prosopis (LeC.)

Sweeping alfalfa and barley: Marana, III-16-55.
Sugar beet: Phoenix, III-18-57.
Rumex sp.: Tucson, III-26-55.

Mimosestes amicus (Horn)

Acacia sp. beans: Tempe, 1953 (E. R. Blakley).
Cercidium floridum beans: Sacaton, V-16-54.
Franseria ambrosiodes: Cave Creek, X-19-57.

Neltumius arizonensis (Schr.)

Olneya tesota blooms: Apache Junction, VI-3-56.

Stator limbatus (Horn)

Cercidium floridum beans: Sacaton, VII-22-54, numerous adults after a 2-month incubation period.

S. pruininus (Horn)

Sweeping cotton: Bowie, VII-14-59.

BUPRESTIDAE

Acmaeodera gibbula LeC.

Heterotheca sp.: Luke Field, IX-18-56.

Melanophila consputa LeC.

Indoors: Phoenix, VII-24-58.

CERAMBYCIDAE

Aethecerinus latecinctus Horn

Cotton sweeping: Pinal Co., July, 1955 (Ken Tipling)

Crossidius sp.

Aplopappus sp.: Sacaton, X-4-54, plentiful.

Derobrachus geminatus LeC.

At light: Phoenix, VII-12-55, plentiful (R. L. Dezember).

CHRYSOMELIDAE

Chaetocnema ectypa Horn, desert corn flea beetle

Baccharis sp.: Tempe, I-5-54.
Barley: Scottsdale, I-7-54.
Corn and sorghum: throughout the state.
Mustard: Phoenix, II-3-54.
Wheat: Peoria, II-28-57, causing blotches on leaves throughout 20-acre field (Dale Berry)

Coscinoptera axillaris LeC.

Olneya tesota: Apache Junction, VI-3-56.

Cryptocephalus sp.

Desert vegetation: Apache Trail, Maricopa Co., X-2-55.

Diabrotica undecimpunctata tenella LeC.

Barley: Scottsdale, I-7-54.
Lettuce: Cochise Co., XI-10-53.

Euryscopa lecontei Crotch

Olneya tesota: Apache Junction, VI-3-54.

Exema globensis Pierce

Franseria ambrosiodes: Cave Creek, X-18-57.

Galerucella xanthomeleana (Schr.), elm leaf beetle

Elm: Phoenix, VI-3-54, severe enough to need control (M. J. Sullivan)

Luperodes melanolomatus Blake

Acacia sp.: Fish Creek Canyon, V-27-56.

Microrhopala rubrolineata (Mann.)

Franseria ambrosiodes: Cave Creek, XI-6-57.

Monoxia sp.

Alfalfa: Phoenix, VIII-31-55.

Oedionychus sp.

Lippia: Buckeye, July, 1954 (Chas. Davis); Phoenix, VIII-14-56 (Frances Schiller).

Pachybrachis spp.

Cotton: Casa Grande, VIII-7-56; Phoenix VII-21-55.
Salix exigua: Fish Creek Canyon, Maricopa Co., XI-9-57 (John Merrick)

Phyllotreta pusilla Horn, western black flea beetle.

Barley: Scottsdale, I-7-54.
Sisymbrium irio: Avondale, I-25-54; Phoenix, II-3-54.

Plagiomorpha arizonae (Crotch)

Salix sp.: Sabino Canyon (Tucson), IX-16-57.

Saxinis scutellaris Schffr.

Cercidium sp.: Tucson, IX-16-57.

Stenopodius nr. *texanus* Schffr.

Sphaeralcea sp.: El Mirage, I-31-54.

S. possibly *martini* Blaisd.

S. sp.: Apache Trail, Maricopa Co., V-27-56.

CLERIDAE

Aulicus femoralis Schffr.

Desert weeds: Phoenix, IX-9-56.

Trichodes ornatus Say

Encelia farinosa: Tempe, IV-9-55 and III-25-57.

COCCINELLIDAE

Brumus desertorum (Csy.)

Cotton: Phoenix, VI-8-55 (Al Caster).

Hyperaspidius pallescens Csy.

Olneya tesota (infested with *Aphis medicaginis*): Apache Junction, V-27- and VI-3-56.

Hyperaspis bensonica Csy.

Pluchea sp.: Phoenix, V-26-55.

H. fastidiosa Csy.

Cotton: Liberty, VIII-14-53 (Bill Zimmer)

H. lateralis wellmani Nun.

Olneya tesota (infested with *Aphis medicaginis*): Apache Junction, V-27- and VI-3-56.

Lindorus lophanthae (Blaisd.)

Tamarix sp. (infested with *Chionaspis etrusca*): Tempe, XI-21-53 (Jack Warren).

Olla v-nigrum plagiata (Csy.)

Pecan (infested with *Monellia costalis*): Maricopa Co., from late summer to frost.

Scymnus apacheanus Csy.

Baccharis sp.: Avondale, I-22-54.
Olneya tesota: Phoenix, V-22-55.
Sphaeralcea sp.: Yuma, V-2-55.

S. loewii Muls.

Indoors: Phoenix, V-13-57.
Sugar beet: Phoenix, V-4-57.

S. sordidus Horn

Indoors: Phoenix, VII-20-55.

Stethorus atomus Csy.

Sugar beet leaves (infested with *Tetranychus cinnabarinus*): Litchfield Park, I-30-58.

CURCULIONIDAE

Anthonomus mimicanus Fall

Aplopappus sp.: Mesa and Tempe, II-16, 17 and 26–54.

A. sphaeralciae Fall

Sphaeralcea sp.: Maricopa Co., entire growing-season.

Apion prob. *occidentalis* Fall

Helianthus sp.: Luke Field A.F.B., X-24-54.

Brachyogmus ornatus Linell

Lycium sp.: Phoenix, V-4-55.

Sphenophorus phoeniciensis Chttn., a billbug

Bermuda grass lawns: Maricopa, Pinal and Yuma Counties, common from spring to fall (Herb Brown and Art Lofgreen).

Centrinaspis acuminatus (Csy.)

Castor bean: Scottsdale, summer of 1956 (Fred Corbus).

C. hospes (Csy.)

Cotton: Liberty, IV-29-54 (Bill Zimmer)

C. penicellus (Hbst.)

Cotton: Cochise Co., July, 1959.

Conotrachelus seniculus LeC.

Cotton: Eloy, VI-6-58 (Skip Hornney); Phoenix, V-24- and VIII-13-54.

Cylindrocopturus adspersus (LeC.)

Cocklebur: Maricopa and Pinal Counties, II-23- and III-3-54. (Bibby & Warren)

Ericydeus lautus LeC.

Pinto bean vine: Cochise Co., VIII-20-54 (C. L. Allen).

Eudiagogus pulcher (Fab.)

Sesbania: Yuma, fall of 1958 (Lew Hammers).

Eupagoderes marmoratus Fall

Desert vegetation: Pinal Co., IX-1-54 (Jack Warren).

Hypera brunneipennis (Boh.), Egyptian alfalfa weevil

Alfalfa: Throughout the state but seldom severe.
Peach (ripening fruit): Tolleson, summer of 1957, several.

Onychobaris pauperella Csy.

Grain sorghum: Toltec, XI-10-53 (Jack Warren).

Macrorhoptus sphaeralciae Pierce

Sphaeralcea sp.: Yuma, V-2-55.

Ophryastes latirostris LeC.

Cotton: Casa Grande, VI-19-56 (R. L. Dezember).

Sitophilus oryzae (L.), rice weevil

Stored barley: Phoenix, VIII-2-55.

Smicronyx imbricatus Csy.

Cotton: Maricopa, VII-23-56; Phoenix, VI-8-55 (Ben Fehrman) and X-7-55; Roll, VI-27-55; Wellton, VI-28-55 and Yuma, VI-20-55.

Trichobaris mucorea (LeC.), tobacco stalk borer

Physalis wrightii and *Solanum elaeagnifolium:* Pinal Co., V-14-54, ovipositing on both host plants (Jack Warren).

Tychius setosus LeC.

Pluchea sericea: Phoenix, V-26-55.

DERMESTIDAE

Anthrenus flavipes LeC.

Clothes closet: Coolidge, V-27-55 (Ken Tipling).

Cryptorhopalum nr. *apicale* Mann.

Mesquite: Luke Field A.F.B., IV-29-56.

Trogoderma granarium Everts, khapra beetle

Grain storage bin: Glendale, XII-5-53, first record in Ariz.

Trogoderma simplex Jayne

Stored strychnine-poisoned oats: Coolidge, II-20-58 (Ken Tipling).

DYTISCIDAE

Thermonectus marmoratus (Hope)

Water: Superstition Mtns., Peralta Canyon, Pinal Co., I-8-54 (Sol Charnofsky).

Laccophilus pictus Cast.

Water: Superstition Mtns., Peralta Canyon, Pinal Co., I-8-54 (Sol Charnofsky).

ELATERIDAE

Anchastus bicolor LeC.

At light: Phoenix, V-6-57.

Aphricus tenuis Fall

Watermelon: Picacho, III-10-54 (Jack Warren).

Conoderus nr. *browni* Knull

Lawn: Phoenix, X-1-56 (Chas. Leake)

Chalcolepidius tartarus Fall

Habitat?: Phoenix, VII-9-54 (Stu Evans).

LYCIDAE

Lycus cruentus LeC.

Pollen: Superstition Mtns., Pinal Co., VIII-14-55.

MELOIDAE

Epicauta alastor Skinner

Cotton: Chandler, VII-8-54.
Host plant?: Queen Creek, VII-17-56 (Irv McMahon).

E. caviceps Horn

Aplopappus sp. (in bloom): Sacaton, X-4-54.
Baccharis sp. (in bloom): Papago Park, Tempe, XI-1-54.

E. ferruginea (Say)

Aplopappus sp.: Sacaton, X-4-54.

E. maculata (Say)

Kallstroemia grandiflora: Adobe, Maricopa Co., IX-15-56.

E. tenella (LeC.)

Host plant?: Queen Creek, VII-17-56 (Irv McMahon).

E. wheeleri Horn

Baccharis sp. (in bloom): Superstition Mtns., Pinal Co., I-8-54.
Aplopappus sp. (in bloom): Papago Park, Tempe, X-16-54.

Lytta auriculata Horn

Lupinus sp.: Tom Mix Monument, Pinal Co., III-26-55.
Sphaeralcea blooms: Phoenix, April, 1954.

L. magister Horn

Desert vegetation: Stanfield, Good Friday, 1955 (Bill Scott).

Lytta mutilata Horn

Dodder: Pinal Co., IX-1 and 3-54 and IV-9-55 (Bibby & Warren).

Negalius marmoratus Csy.

Desert vegetation: Luke Field A.F.B., IX-8-56; Red Rock, IX-1-54 (Bibby & Warren).

Nemognatha sp.

Host plant?: Cochise Co., VII-19-59 (Ben Fehrman).

Tegrodera latecincta Horn

Alfalfa field: Maricopa, May, 1957 (Chas. Ash).

NITIDULIDAE

Carpophilus hemipterus (L.)

Grain sorghum: Toltec, Pinal Co., XI-10-53 (Jack Warren).

Conotelus mexicanus Murray

Cotton blooms and other blooms: Throughout state.

Cybocephalus sp.

Atriplex sp.: Avondale, I-3-54.

SCARABAEIDAE

Cotalpa consobrina Horn

At light: Phoenix, VII-27-55, numerous.

Cotinis nitida (L.), green June beetle

Cotton bloom: Chandler, VIII-18-55 (Joe Hartsig).

Cyclocephala sp., probably desert June beetle

Host plant?: Chandler, VII-3 and 25-54; Toltec, VIII-6-54.

Diplotaxis brevicollis LeC.

At light: Payson, VII-4-59 (Chas. Ash).

D. cribulosa LeC.

Peach foliage: Phoenix, VII-21-59, causing damage (Walter Brown).

D. obscura LeC.

At light: Payson, VII-4-59 (Chas. Ash).

Oxygrylius ruginosus LeC.

At light: Red Rock, IX-1-54 (Jack Warren).

Phyllophaga serofaria LeC.

At light: Phoenix, VII-3-56 and VII-17-59.

P. timida Horn

At light: Cave Creek, September, 1957 (Marion Cox).

Plusiotis gloriosa LeC.

Host plant?: Camp Verde, summer, 1958 (Randall Ramsey).

STAPHYLINIDAE

Platystethus sp.

Cotton: Buckeye, VIII-6-53 (Bill Zimmer)

P. spiculus Er.

Dry ice: Vegetable packing house, Somerton, VI-20-55, numerous.

TENEBRIONIDAE

Alphitobius diaperinus (Panzer)

Chicken house: Phoenix, V-12-59, a nuisance.

Blapstinus sp.

Alfalfa: Yuma, II-10-54 (Don McCain).
Soil: Avondale, I-29-54 (Bill Zimmer).

Edrotes ventricosus LeC.

Cotton field: Maricopa, II-2- and III-21-58 (Herb Brown).

Eleodes obscura sulcipennis Mann.

Pasture: Camp Verde, X-20-57.

COLLEMBOLA

ENTOMOBRYIDAE

Drepanocyrtus sp.

Alfalfa field: Buckeye, I-31-55 (Bill Zimmer) and III-17-55 (Chas. Davis) and Queen Creek, II-4-55 (Ken Tipling).

Entomobrya sp.

Alfalfa field: Queen Creek, II-4-55 (Ken Tipling).

Proistoma minuta Tullberg

Onion field: Litchfield Park, II-2-56 (Zimmer).

Pseudosinella violenta rolfsi Mills

Cornstalk: Gilbert, VIII-12-56, within tunnel of *Zeadiatrea grandiosella* (Dyar) southwestern corn borer.

DERMAPTERA

LABIDURIDAE

Euborellia cincticollis (Gerst.), an African earwig (Nutting 1960)

Cantaloupe: Yuma, 1954 (Don McCain) and V-22-57.
Corn stalk: Gilbert, VIII-12-56, within tunnels of *Zeadiatrea grandiosella* (Dyar), southwestern corn borer.
Grain sorghum stubs: Casa Grande, II-18-59 and Gilbert II-22-57 (Bibby & Merrick).

Labidura riparia (Pallas)

Bermuda grass: Dome, X-30-55 (Bibby & Brown).
Hotel patio: Yuma, X-21-56.
Indoors: Phoenix, X-31-56.
Soil: Maricopa, I-9-58 (Chas. Ash).

DIPTERA

AGROMYZIDAE

Liriomyza spp., serpentine leaf miner

Melons and some other crops: A serious pest in various sections of the state.

CALLIPHORIDAE

Eucalliphora lilaea (Walk.)

Automobile: Avondale, I-29-54.

CHLOROPIDAE

Oscinella frontoorbitalis Sabr.

Johnson grass: Phoenix, I-24-54.

DOLICHOPODIDAE

Pelastoneurus dissimilipes Wheeler

Small pond: Picacho, X-25-53 (Jack Warren).

MUSCIDAE

Hylemya cilicrura (Rond.), seed-corn maggot

Various crops: Throughout state, often severe. There was one instance of its being found in lettuce stems of a commercial planting at Beardsley, Maricopa Co., II-27-56 (Ben Fehrman) where it caused many of the plants to wilt and die.

TACHINIDAE

Exorista larvarum (L.)

Salt-marsh caterpillar: Avondale, I-7-54, from pupae of salt-marsh caterpillar collected XII-30-53 (Bill Zimmer)

Phorocera sp.

Salt-marsh caterpillar: Same as above for *E. larvarum*.

Phorocera nr. *floridensis* Tns.

Pupa of *Pieris protodice* (southern cabbageworm): Goodyear, X-15-55.

Voria ruralis (Fall.)

Pupae of *Trichoplusia ni* (cabbage looper): Phoenix, XI-5-53 (Bibby & Hammers) and Picacho, X-26-53 (Jack Warren).

SYRPHIDAE

Eumerus sp., probably lesser bulb fly

Iris bulbs: Phoenix, XI-26-57, causing damage.

Tubifera tenax (L.)

Manure in corral: Yuma, XII-7-58 (Lew Hammers).

TETHINIDAE

Pelomyia coronata (Lw.)

Young lettuce: Eloy, III-12-56 (Spitler & Hornney).

TEPHRITIDAE

Evarestoides acutangula (Thoms.)

Pluchea sericea: Avondale, I-29-54.
Sisymbrium irio: Avondale, I-25-54.

Zonosemata electa (Say)

Solanum elaeagnifolium: Toltec, Pinal Co., XI-10-53, larvae within fruit (Jack Warren).

Trupanea stigmatica (Coq.)

Franseria deltoidea: Tempe, II-57-54.

EMBIOPTERA

OLIGOTOMIDAE

Oligotoma nigra Hagen

Indoors at light: Maricopa and Pinal Counties, VII-17-54 and V-4-58.
Frost-bitten Johnson grass: Phoenix, I-28-54.

HETEROPTERA

ANTHOCORIDAE

Anthocoris borealis Dallas

Poplar gall: Pinal Co., IV-22-55, nymphs and adults in association with aphids and thrips.

Orius tristicolor (White)

Various crops and other plants: Throughout the state; a predator especially fond of thrips.

Xylocoris flavipes (Reuter)

Stored grain: Eloy, III-12-56 (G. H. Spitler)

COREIDAE

Acanthocephala thomasii (Uhler)

Desert vegetation: Apache Trail, Maricopa Co., X-9-55 (R. L. Dezember).

Arhyssus lateralis (Say)

Sphaeralcea sp.: Queen Creek, III-9-54.
Chenopodiaceous shrub: Eloy, XI-20-54.

Catorhintha selector Stål

Boerhaavia sp.: Apache Trail, Maricopa Co., X-2-55.

Mozena arizonensis Ruckes

Cotton: Phoenix, VI-16-58 (Stu Evans) and VII-13-58 (Berry & Bibby); and Queen Creek, VI-11-58 (Irv McMahon).

Scolopocerus uhleri Dist.

Parked automobile: Phoenix, X-15-55, numerous.

EMESIDAE

Aknisus multispinosus Ashm.

Suaeda torreyana: Avondale, XI-20-53 (Bill Zimmer).

LYGAEIDAE

Geocoris punctipes (Say)

Atriplex sp.: Glendale, VI-13-54.

Ligyrocoris auricillianus (Dist.)

Cotton: Marana, V-14-56.

Lygaeus analis Dall.

Simmondsia chinensis: Superstition Mtns., VIII-14-55.

L. belfragei Stål

Wislizenia refracta: Goodyear, III-1-54.

L. kalmii Stål, small milkweed bug

Alfalfa: Stanfield, V-23-56 (Herb Brown).
Bermuda grass: Cave Creek and Phoenix, VI-1 to 8-57 (Bibby & Zimmer).
Cotton: Coolidge, July, 1955 (Bibby & Tipling).

Melanocoryphus lateralis (Dallas)

Cotton: Bowie, VII-14-59 (John Robertson), of no economic importance.

Nysius ericae (Schill.), false chinch bug

Baccharis sp.: Avondale, I-3 and 22-54; Tempe, I-5-54.
Sphaeralcea sp.: Litchfield Park, I-24-54.

N. monticola Dist.

Quercus sp.: Prescott, X-31-54.

Harmostes angustatus Van D.

Chenopodium sp.: Glendale, X-24-54 (Sol Charnofsky)

H. reflexulus (Say)

Actinea odorata: Maricopa, IV-9-56.
Aplopappus sp.: Sacaton, X-4-54.
Baileya multiradiata: Apache Junction, Maricopa Co., IV-10-55.
Cotton: Chandler, July, 1954 (Irv McMahon); Cochise Co., VII-14-59; of no economic importance.
Lettuce: Phoenix, III-24-54.

Leptocoris trivittatus (Say)

Orchard: Cochise Co., Feb., 1954 (Clint Allen).

Leptoglossus zonatus (Dallas)

Around building: Congress, II-13-55 (J. L. Stewart).
Citrus: Phoenix, X-8-53 (Ted Swift).

Liorhyssus hyalinus (F.)

Cercidium sp.: Tempe, XI-9-54.
Grain sorghum: Toltec, Pinal Co., XI-10-53.
Lettuce: Phoenix, X-11-54; Yuma, VI-12-57, damageable infestation (Lew Hammers).
Manzanita: Prescott, X-31-54.
Sisymbrium irio: Scottsdale, I-7-54.
Tamarix sp.: Tempe, XI-21-53.

Narnia pallidicornis Stål

Cactus: Toltec, Pinal Co., IV-30-56 (Dick Leigh).

Niesthrea sidae (F.)

Abutilon sp.: Peralta Canyon, Superstition Mtns., Pinal Co., X-24-54 (E. R. Blakley), adults and nymphs numerous.

N. raphanus How.

Cotton: Phoenix, III-28-56 (Fred Corbus).
Citrus: Maricopa Co., spring, 1956, young trees damaged.
Lettuce: Cochise Co., XI-10-53.
Sisymbrium irio: Pima, Pinal and Maricopa Cos., spring, 1956, heavy infestations.
Tamarix tree: Tolleson, VI-7-57, thousands of specimens on trunk, twigs and straw.

N. tenellus Barber

Safflower: Mesa, IV-7-59, numerous (Chas. Ash).

MIRIDAE

Adelphocoris superbus (Uhler)

Cotton: Cochise Co., VIII-20-54 (Allen & Bibby), not recorded from other sections of state.

Atomoscelis modestus (Van D.)

Chenopodium sp.: Tolleson, VI-7-57.

Chlamydatus suavis (Reut.)

Ambrosia artemisiifolia: Roll, VI-27-55 (Bibby & Brown).

Cyrtopeltis modestus (Dist.), a suckfly

Tomato (volunteer): Gila River, Avondale, X-17-54 (Charnofsky & Bibby).

Dichrooscytus nr. *sequoiae* Bliven

Rose: Phoenix, IV-4-55 (R. L. Dezember)

Halticotoma valida Reut.

Yucca sp.: Cave Creek, III-30-58 (Chas. Ash), numerous.

Lopidea eremita Van D.

Olneya tesota: Tempe, V-17-54 (Bibby & Hartsig)

Lygus desertus Knight

Baccharis sp.: Pinal Co., I-14-54.

Lygus spp.

Alfalfa seed, cotton and other crops: Many sections of state, serious pests, necessitating control measures.

Melanotrichus nicholi Knight

Sphaeralcea sp.: Tempe, III-30-57.

Neurocolpus arizonae Knight

Mesquite: Eloy, IV-28-54, Luke Field A.F.B., IV-29-56.

Parthenicus aureosquamis Knight

Cotton: Coolidge, VII-23-55, seen occasionally (Ken Tipling); Phoenix, VIII-13-58 (Berry & Bibby), suspected of damaging tiny squares (flower buds) as does the cotton flea hopper, *Psallus seriatus.*

P. baccharidis Knight

Baccharis sp.: Avondale, I-3-54.

Phytocoris vividus (Uhler)

Larrea tridentata: Tempe, IV-9-55, numerous.

Psallus flora Van D.

Wislizenia refracta: Pinal Co., V-4-58.

P. seriatus (Reut.)

Cotton: All sections of State, a pest, sometimes serious.
Tidestromia lanuginosa: Scottsdale, X-16-54, suspected of laying overwintering eggs.

Rhinacloa forticornis Reut.

Alfalfa: Phoenix, X-17-55.
Aplopappus sp.: Sacaton: X-4-54.
Chenopodium sp.: Glendale, X-24-54.
Cotton: Perryville, VI-29-54 (Bill Zimmer). It was commonly called black fleahopper and often became a pest of cotton.
Franseria ambrosiodes: Cave Creek, X-19-57.
Hyptis emoryi: Picacho, XI-22-54 (Bibby & Warren).
Malva parviflora: Scottsdale, I-7-54.

Spanogonicus albofasciatus (Reut.)

Cotton: Maricopa Co., 6-25-54, a pest doing damage similar to that of *Psallus seriatus* and *Rhinacloa forticornis.*
Lettuce: Cochise Co., XI-10-53.
Populus sp.: Pinal Co., IV-1-58.
Tidestromia lanuginosa: Phoenix, VIII-3-55.

Trigonotylus n. sp.

Bermuda grass: Dome, Yuma Co., VI-27-55, numerous (Brown & Bibby).

Tuponia sp.

Atriplex sp.: Tempe, I-5-54.

NABIDAE

Nabis alternatus Parshley

Barley: Scottsdale, I-7-54.
Sisymbrium irio: Coolidge, I-10-54 (Bibby & Warren); Phoenix I-11-54; Scottsdale, I-7-54.

N. ferus (L.)

Alfalfa: Yuma, II-10-54.
Lettuce: Queen Creek, XI-26-53 (Bibby & Tipling)
Sphaeralcea sp.: Luke Field A.F.B., II-21-54.
An additional species of *Nabis* is recorded by Werner & Butler (1957).

PENTATOMIDAE

Aethus conformis (Uhler)

Indoors: Phoenix, VII-23-54 (M. F. Wharton)

Brochymena pilatei Van D.

Elm: Yuma, IX-9-53 (Don McCain).
Salix babylonica: Peoria, VII-5-57 (Ben Fehrman).
Weeds: Liberty, Maricopa Co., VII-21-54.

Camirus moestus (Stål)

Franseria ambrosiodes: Cave Creek, X-19-57.

Chlorochroa ligata (Say), conchuela

Barley: Pinal Co., III-30-56.
Cotton: Chandler, VIII-18-55 (Bibby & Hartsig); Queen Creek, VII-10-57 (Irv McMahon)

C. sayi Stål, Say stink bug

Potato: Queen Creek, V-21-56.
Sphaeralcea sp.: Coolidge, V-1-56.
Sugar beet: Phoenix, VI-7-57, heavy infestation.
Wislizenia refracta: Goodyear, X-5-55.

C. uhleri Stål

Wheat: Yuma, V-2-55.

Corimelaena incognita (McA. & M.)

Atriplex sp.: Eloy, VI-10-54.
Nicotiana sp.: Buckeye, V-18-57 and Tucson, V-23-55.

Euschistus servus impictiventris Stål

Cotton: Buckeye VIII-20-57 and Phoenix VII-21-55.
Lettuce: Roll, XI-1-55 (Bibby & Brown).
Soybean: Goodyear, IX-23-55, heavy infestation.

E. servus servus (Say), brown stink bug

Sugar beet: Mesa, V-12-55.

Podisus acutissimus Stål

Alfalfa: Phoenix, VII-16-55.
Cotton: Stanfield, VIII-23-57 (Herb Brown).

Sphyrocoris punctellus (Stål)

Gossypium thurberi: Superstition Mtns., Pinal Co., X-24-54 (E. R. Blakley) and XI-14-54 (Bibby & Warren).

Thyanta brevis Van D.

Lycium sp.: Phoenix, V-4 and 29-55.
Suaeda torreyana: Estrella Park, Goodyear, VII-5-57 (Chas. Ash).
Sugar beet: Phoenix, V-19-57.

T. custator of authors not (F.)

Aplopappus sp.: Glendale, X-24-54 and Tempe, X-16-54.

Bermuda grass: Roll, VI-25-55 (Bibby & Brown).
Suaeda torreyana: Goodyear, X-5-55.

T. pallidovirens spinosa Ruckes

Cotton: Phoenix, III-6-57.

T. rugulosa (Say)

Suaeda torreyana: Goodyear, X-5-55.

PIESMIDAE

Piesma cinerea (Say)

Suaeda torreyana: Avondale, XI-26-53.

P. rugulosa McA.

Atriplex sp.: Sacaton, VI-4-54.

PYRRHOCORIDAE

Arhaphe cicindeloides Walker

Sand along stream: Oak Creek, IX-6-54 (Jack Warren). Specific name justified by habitat and superficial appearance. Recently it received publicity because of an apparent stridulatory structure (Lattin 1958).

Largus cinctus H.-S.

Alfalfa: Pinal Co., I-5-56; Stanfield, VI-12-56, nymphs and adults seen frequently (Herb Brown).
Corn: Stanfield, VI-15-56 (Herb Brown).
Cotton: Queen Creek, July, 1957 (Irv McMahon) and in other sections of the state rarely doing damage and necessitating control measures.
Desert vegetation: Apache Trail, Maricopa Co., X-2-55.
Small grain: Eloy, II-22-56 (Bibby & Hornney).

REDUVIIDAE

Apiomerus sp.

Various plants: Cochise Co., not so common as other predaceous species of reduviids listed below.

Sinea confusa Caudell

Beets and weeds: Phoenix, X-7-53, and at other places on cultivated crops from time to time.

Zelus renardii Kol.

Alfalfa: Yuma, II-10-54 (Don McCain).
Baccharis sp.: Avondale, I-22-54.

Z. socius Uhler

Aplopappus sp.: Sacaton, X-4-54.
The two species of *Zelus* were definitely more prevalent than either of the other two species of reduviids listed above. Additional reduviids were recorded by Werner & Butler (1957).

HOMOPTERA

ALEYRODIDAE (supplement to list by Bibby (1959)).

Aleuroplatus ovatus Q. & B.

Barberry leaves: Yavapai Co., XI-8-58.

CERCOPIDAE

Clastoptera arizonana Doering

Baccharis sp.: Luke Field A.F.B., June, 1955.
Cercidium microphyllum: Tempe, XI-1-54.
Mullein: Camp Wood, X-31-54.
Olneya tesota: Apache Junction, VI-3-56; Tempe, V-25-55.

CICADIDAE

Diceroprocta apache Davis

Tamarix sp.: Maricopa, Pinal and Yuma Cos., July, 1959, numerous, ovipositing in cotton plants at some places.

Tibicen resh Hald.

Populus sp.: Camp Verde, IX-14-58.

COCCOIDEA

Antonina graminis (Mask.) Rhodes-grass scale

Bermuda grass: Phoenix, I-9-59 (Joe Hartsig).

Chionaspis etrusca Leon., tamarisk scale

Tamarix sp.: Maricopa Co., XI-21 to 23-53 (Bibby & Warren), general according to Hopkins & Carruth (1954).

Coccus hesperidum L., soft scale

Grapefruit: Peoria, X-5-55, several trees heavily infested (Ben Fehrman).

C. pseudomagnoliarum (Kuw.), citricola scale

Citrus: Peoria, IV-9-59, few trees heavily infested (Stu Evans).

Diaspis echinocacti (Bouché), cactus scale

Opuntia fragillis: Tempe, 1953 (E. R. Blakley).

D. simmondsiae Ferris

Simmondsia chinensis leaves: Superstition Mtns., Pinal Co., VIII-14-55.

Diaspis toumeyi Ckll.

Holacantha emoryi: Pinal Co., II-13-56 (Bibby & Hagerty).

Dactylopius confusus (Ckll.)

Opuntia sp.: Cave Creek, IX-13-59 (Bibby & Chilton).

Icerya purchasi Mask., cottony-cushion scale

Citrus, pittosporum and other ornamentals: Statewide, occasional infestation; biological control with vedalia practiced.

Lecanium corni Bouché, European fruit lecanium

Plum twig: Mesa, IV-8-57, severe (P. D. Gerhardt).

Lichtensia lycii Ckll.

Lycium sp.: Litchfield Park, IV-9-57; Papago Park, Tempe, IV-9-55.

Odonaspis ruthae Kot.

Bermuda grass: Statewide and serious pest of Bermuda grass seed-crop in Yuma Co.

Orthezia annae Ckll.

Atriplex salt bush: Yavapai Co., VIII-17-58.

Phenacoccus solenopsis Tinsley, a mealybug

Cotton: Luke Field A.F.B., VII-14-55, several acres of 1st year (not sucker) cotton severely infested (Zimmer & Bibby). This mealybug is listed by Fernald (1903) as having been collected in nest of *Solenopsis geminata* in New Mexico with no other record of it. However, she recorded *P. gossypii* on cotton and other mallows.

Pseudodiaspis yuccae (Ckll.)

Cercidium microphyllum: Mesa, XII-54-53.

Puto lasiorum (Ckll.)

Simmondsia chinensis: Superstition Mtns., Pinal Co., VIII-14-55.

Saissetia oleae (Bern.), black scale

Cotton: Buckeye, VII-6-58, severe on several acres (Bibby, Davis & Wene).
Fig leaves and twigs: Phoenix, X-9-56 (Dick Hubbs).
Oleander: Mesa, X-14-55 (L. W. Sheets), infestation light.

Tachardiella fulgens (Ckll.)

Coursetia microphylla: Sabino Canyon, Tucson, IX-16-57.

DELPHACIDAE

Delphacodes campestris Van D.

Beets and weeds: Phoenix, X-7-53 (Bibby & Ritchie).
Lettuce: Queen Creek, XI-26-53.

HYMENOPTERA

ARGIDAE

Sofus pilicornis (Hohngr.), a sawfly

Cantaloupe: Yuma, IV-21-56 (Lew Hammers), collected on.
Purslane (portulaca): Yuma, IX-9-53 (Bibby & McCain), ravenously devouring the weed.

BETHYLIDAE

Cephalonomia tarsalis (Ashm.)

Stored grain: Eloy, III-12-56 (G. H. Spitler).

BRACONIDAE

Agathirsia testacea Muesb.

Larva of *Acontia cretata:* Yuma, VIII-19-53.

Lysiphlebus testaceipes (Cress.)

Various aphids: Statewide.

Bracon gelechiae Ashm.

Chionodes sistrella and/or *Coleophora* larvae: Avondale, XI-26-53.

CHALCIDIDAE

Brachymeria sp.

Pieris protodice pupae: Goodyear, X-5-55.

EURYTOMIDAE

Bruchophagus gibbus (Boh.), clover seed chalcid

Alfalfa seed crop: Statewide and at times a serious pest.

Spilochalcis flavopicta (Cress.)

Pieris protodice: Goodyear, X-5-55, possibly a secondary parasite.

CYNIPOIDEA

Ganaspidium sp.

Aphid-infested oleander: Phoenix, IV-18-59, in rearing jar with parasites of *Aphis nerii.*

ENCYRTIDAE

Aphidencyrtus aphidivorus (Mayr)

Suaeda torreyana (aphid- and psyllid-infested): Avondale, XI-26-53. Secondary parasite of aphids.

EULOPHIDAE

Aphelinus nigritus How.

Hysteroneura setariae (rusty plum aphid): Phoenix, XI-6-55, aphids heavily parasitized.

Closterocerus utahensis Cwfd.

Alfalfa (aphid- and probably leaf miner-infested): Pinal Co., I-11-56 (Bibby & Brown).
Gossypium thurberi (bucculatrix-infested): Peralta Canyon, Pinal Co., XI-14-54 (Bibby & Warren).

Derostenus agromyzae Cwfd.

Atriplex lentiformis (seed and twigs): Tempe, X-4-53.

Encarsia meritoria Gahan

Ambrosia (whitefly- and tingid-infested): Luke Field A.F.B., X-16-55.

Eretmocerus haldemani Howard

Ambrosia (whitefly- and tingid-infested); Luke Field A.F.B., X-16-55.

Galeopsomyia columbiana (Ashm.)

Suaeda torreyana (aphid-, psyllid- and caterpillar-infested): Avondale, XI-26-53.

ICHNEUMONIDAE

Diplazon laetatorius (F.) a parasite of syrphid flies

Alfalfa field: Pinal Co., I-5-56.
Sugar beet field: Phoenix, V-25-57.

PTEROMALIDAE

Catolaccus aeneoviridis (Grlt.), a secondary parasite

Pieris protodice pupae: Goodyear, X-5-55.

C. victoria Burks, prob. a secondary parasite

Atriplex seed: Tempe, X-14-53.

Pachyneuron allograptae Ashm.

Puparium of syrphid fly: Mesa, spring, 1959 (R. L. Dezember).

P. siphonophorae (Ashm.), secondary parasite of aphids

Aphid-infested grain sorghum: Avondale, X-23-53 (Bibby & Zimmer).

THYSANIDAE

Thysanus niger (Ashm.), a secondary parasite

Psyllid: Maricopa, VII-26-56.

TIPHIIDAE

Myzinum frontale Cr.

Cotton field: Buckeye, VI-24-54 (Chas. Davis); Roll, VI-27-55 (Bibby & Brown).

M. maculatum (F.)

Cotton field: Marana, VI-10-54 (Jack Warren).

TORYMIDAE

Microdontomerus anthonomi (Cwfd.)

Pupae of *Pieris protodice:* Goodyear, X-5-55.

Liodontomerus insuetus Gahan

Bruchid-infested cercidium beans: Sacaton, V-16-56.

TRICHOGRAMMATIDAE

Trichogramma minutum Riley

Eggs of *Estigmene acrea:* Phoenix, Arizona, VIII-12-55.

VESPIDAE

Polistes exclamans Viereck

Citrus groves and residential ornamentals: Salt River Valley, a nuisance because of its painful and venomous sting. The biology and control of the pest were given by Hopkins (1955).

LEPIDOPTERA

ARCTIIDAE

Estigmene acrea (Drury), salt-marsh caterpillar

Cotton and other crops: Statewide and a serious pest, noticeably hampered in the fall of 1958 by a fungus disease of the larvae determined as *Entomopthora aulicae* (Reich.) by C. G. Thompson.

COLEOPHORIDAE

Coleophora sp.

Suaeda torreyana: Avondale, XI-26-53 and Maricopa VII-24 & 28-56, within cottony-covering of a psyllid.

CRAMBIDAE

Chilo loftini Dyar, a borer

Grain sorghum: Phoenix, IX-20-54 (Stu Evans); Casa Grande, VII-24-56 (R. L. Dezember)
Johnson grass: Mesa, VIII-5-59 (John Chilton).
Wheat stem: Phoenix, V-4-56 (Bill Ritchie).
This borer was reported by Glick (1922) in volunteer sugarcane at Marinette, Maricopa Co.

Zeadiatrea grandiosella (Dyar), southwestern corn borer.

Corn and sorghum: A serious pest in many sections of state.

GELECHIIDAE

Chionodes sistrella (Busck)

Suaeda torreyana: Avondale, XI-20-53 (Bill Zimmer) and Maricopa, VII-24 & 28–56, larvae within cottony-covering of a psyllid.

Kieferia lycopersicella (Busck), tomato pinworm

Tomato: Yuma, June, 1954 (Don McCain).

Pectinophora gossypiella (Saund.), pink bollworm

Cotton: Buckeye—Gila Bend: VII-1-58 (Chas. Davis), the first infestation found in Maricopa Co. in several years.

HELIOZELIDAE

Coptodisca sp.

Cotton leaves: Phoenix, VII-13-56 (Al Caster), flat, seed-like pupa which had transformed from larva that had dropped or had been blown from leaves of cottonwood (*Populus fremontii*), its true host.

LASIOCAMPIDAE

Malacosoma fragile (Stretch), a tent caterpillar

Populus fremontii: Maricopa Co., III-4-58, common.

LYONETIIDAE

Bucculatrix thurberiella Busck, cotton leaf perforator

Cotton: Statewide, beginning midseason, a serious pest.

Gossypium thurberi: Peralta Canyon, Pinal Co., XI-14-54 (Bibby & Warren).

NOCTUIDAE

Acontia cretata (Grt. & Rob.)

Sphaeralcea sp. flower buds: Yuma, V-2-55, larvae feeding ravenously.

Sida hederaceae: Yuma, VIII-19-53; listed from *Abutilon pedunculare* in Texas and/or Mexico by Moreno & Bibby (1943).

Alabama argillacea (Hbn.)

Cotton: Various places from year to year, seldom of economic importance.

Thurberiphaga diffusa (Barnes), thurberia bollworm

Gossypium thurberi (thurberia): Sabino Canyon, Pima Co., IX-16-57 (Ash & Bibby), closely restricted to thurberia and not a potential pest of cotton according to Vorhies (1926).

NYMPHALIDAE

Vanessa cardui (L.), painted lady

Hollyhock: Phoenix, II-16-54; occasional outbreaks of larvae on cotton and other crops.

PAPILIONIDAE

Papilio cresphontes Cram., orange-dog

Orange leaves: Phoenix, XI-13-54, not a serious pest of citrus in Arizona.

PHYCITIDAE

Elasmopalpus lignosellus (Zell.), lesser cornstalk borer

Grain sorghum, soybeans, and Johnson grass: various sections of state, a severe pest of grain sorghum planted in June and July, sometimes it lessened stands to such an extent that the crop was replanted or abandoned. Johnson grass served as an overwintering host from which it spread to cultivated crops, both sorghum and soybeans.

Homoeosoma electellum (Hlst.), sunflower moth

Sunflower heads: Queen Creek, VII-8-57 (Irv McMahon), severe infestation of larvae on commercial crop.

PYRAUSTIDAE

Noctuelia rufofascialis (Stph.)

Sphaeralcea sp.: Scottsdale, IV-26-56, larvae numerous in flower buds; recorded from Mexico and/or Texas from cotton and many other mallows by Moreno & Bibby (1943).

Loxostege similalis (Guen.), garden webworm

Various crops and weeds: Statewide, seldom a serious pest.

Udea rubigalis (Guen.), celery leaf tier

Sugar beet: Tolleson, II-20-59 (Ash & Ritchie).

SPHINGIDAE

Celerio lineata (Fab.), white-lined sphinx

Desert and mountain vegetation: Camelback Mtns., VIII-16-55, frequently spreaded from desert vegetation to cultivated crops.

TORTRICIDAE

Platynota stultana (Wlsm.), a leaf roller

Cotton, melons, sugar beet and other crops: Statewide, a serious pest.

PSOCOPTERA

LACHESILLIDAE

Lachesilla rena Somm.

Grain sorghum head: Phoenix, XI-5-53 (Bill Ritchie).

LIPOSCELIDAE

Liposcelis bostrycophilus complex
L. rufus Bdhd.
L. n. sp.

Frost-bitten Johnson grass: Phoenix, I-24-54.
Stored barley: Pinal Co., IX-15-54 (Ken Tipling).

THYSANOPTERA

(a supplement to list by Bibby (1958))

AEOLOTHRIPIDAE

Dactuliothrips boharti Bailey

Phacelia sp.: Amphitheater, Papago Park, Maricopa Co., IV-5-58, not recorded from this host by Bailey (1957) and furthermore it is probably the first record of the thrips being in Arizona.

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Fluorescent Dye Technique for Studying Distribution of Spray Oil Deposit on Citrus[1]

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ABSTRACT

An oil soluble fluorescent dye was incorporated with various petroleum oils to provide a qualitative means of studying the relative deposit of the dilute oil spray mixtures on various parts of grapefruit trees on exposure under ultraviolet light. This technique provided a good means of qualitative comparisons of differences in oil deposit associated with (1) the concentration of oil in the spray mixture, (2) various surfactants and (3) concentration of surfactant. The dye penetrated with the oil into the outer mesocarp of the green fruit with applications during July while less penetration was observed from those made during August. Only surface deposit appeared to have occurred on mature grapefruit held in storage from the 1959 crop. The dye penetrated into the bark of green twigs but appeared to have been deposited only on the surface of the bark of small branches. The dye appeared to have penetrated into the leaf mainly at the midrib and larger veins. Photographs of petroleum oil deposit on the fruit, leaves, and twigs and small branches are included.

The deposit of petroleum spray oil can be determined quantitatively from selected measured areas of tree foliage or fruit, but the amount of deposit may be in no way related to the distribution or nature of the film of the oil on the particular plant part. A rapid qualitative means of comparing the petroleum oil deposit on various parts of the citrus tree became a very important consideration in an investigation for the development of petroleum spray oils for use on citrus in the Rio Grande Valley area of Texas. Petroleum oils are applied to citrus in this area for the control of diaspid scales which move only during a short time early in their growth period. The species of importance generally attack all parts of the citrus tree.

Differential control of California red scale (*Aonidiella aurantii* (Maskell)) has been found on various plant parts. Ebeling (1936) reported the greatest difficulty in the control of scale insects was on the bark of citrus trees because of the absorption of oil by the rough bark and possibly also because of a lighter deposit of oil caused by the nature of the surface of the bark of large branches of citrus trees. Ebeling (1939) also suggested that the thin film of oil applied to the branches upon which the scales rest is absorbed by the bark so rapidly that the oil often does not have sufficient time to penetrate the mass of loose wax threads under the scale, which blocks movement of the oil to the spiracles.

The relation of the concentration of surfactant to the amount of oil deposited on grapefruit as determined by procedures similar to those of Riehl *et al.* (1953) was investigated in laboratory studies. While application of the data then made it possible to produce chosen levels of deposit of spray oils on grapefuit, it was desirable to know whether the various oils were deposited uniformly on all parts of the tree. Quantitative determinations of amounts of deposit on various plant parts would involve a large investment of time. A method was developed to incorporate a fluorescent dye in the spray oil for the purpose of observing the deposit of oil left by the spray mixture on various plant parts as indicated by the fluorescence of the dyed oil, visible on exposure to ultraviolet light. Such a technique was thought to have considerable application in the selection of surfactants for possible use with various petroleum fractions as citrus spray oils and oils used on other tree crops. A search of the literature failed to provide information on a dye suitable for this purpose.

MATERIALS AND METHODS.—*Selection of Fluorescent Dye.*—A number of dyes were tested in order to select one that was soluble in oil and insoluble in water, and produced an intense visible fluorescence when exposed to 2537 Angstrom unit (Å) wave length ultraviolet light. These requirements were necessary because after spraying citrus with dyed-oil in water mixtures, any fluorescence observed on the citrus must be the result of deposited oil. If the dye were even slightly soluble in water, it would not be possible to determine whether the fluores-

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Table 1.—Dyes tested for solubility.

Calco Alizarine Cyanine Green Base[a]	Fluorescent Green NAC[b]
Calco Fluorescein[a]	Fluorescent Orange[b]
Calcofluor Sea Green[b]	Fluorescent Yellow C-4[b]
Calcofluor White LP Conc.[b]	Fluorol OB[b]
Calcofluor White RW[a]	Fluorol OBR[b]
Calcofluor Yellow HEB[b]	Marker VL[b]
Fluorescent Blue No. 3[b]	Oil Color No. 48[b]
Fluorescent Fire Red[b]	Oil Color No. 131[b]

[a] Samples provided for testing through the courtesy of the American Cyanamide Company.
[b] Samples provided for testing through the courtesy of the Ethyl Corporation.

cence indicated the location of oil or of water. A list of the dyes tested is presented in table 1.

Fluorescent Yellow C-4 was found to best satisfy the requirements for the dye. It gave an intense yellow fluorescence when dissolved in oil, and in the presence of ultraviolet light. It did not fluoresce in the solid (undissolved) state. This dye is manufactured by Patent Chemicals, Inc., Paterson, New Jersey. Fluorescent Yellow C-4 is an anthraquinone derivative, belonging to the series 2-alkylamino-1,9-methyl pyridino anthrone. The manufacturer stated that no study had been made of the toxicological properties on this particular dye. However, because of their many years of experience with related dyes, they believed the dye to be relatively inert and completely safe to handle, using ordinary precautions.

The term "fluorescence" signifies the ability of the dye to absorb short wave length light and emit or radiate light of a longer wave length. The radiated light from Yellow C-4 Dye was in the visible spectrum, and since it fluoresced bright yellow it had a wave length of about 6000 Å. Both 3660 Å and 2537 Å ultraviolet lamps were considered for use as a source of short wave length light. Yellow C-4 Dye gave a more intense fluorescence with the 2537 Å light. Also, since it was desirable to photograph only fluoresced light, the shorter wave length source was considered the more appropriate of the two. Scattered and reflected light of 3660 Å is transmitted by ordinary camera lens whereas the 2537 Å light is not transmitted (Anonymous 1955). The camera lens, therefore, would act as a filter for the latter ultraviolet light. In addition, special filters, subsequently discussed, were used to absorb the small amount of longer wave length ultraviolet light and visible violet light emitted by the light source.

Preparation of Oil Samples.—Powdered Yellow C-4 Dye was added to each of the oils used in this study. About 0.25 gram of dye was used for each 100 grams of oil. The mixtures were machine shaken for about 6 hours. During this period they were heated twice to 140° F. Excess dye was allowed to settle. The dyed oil was filtered twice through a medium porosity filter paper. In those cases where it was desirable to know the dye content in the oil, the determination was made using a recording spectrophotometer. The color density of the dyed-oil was such that it was necessary to reduce the absorption of the sample. To accomplish this, cell spacers were used to reduce the path length to 1 mm. The dye content of the sample was established by comparing its absorption curve

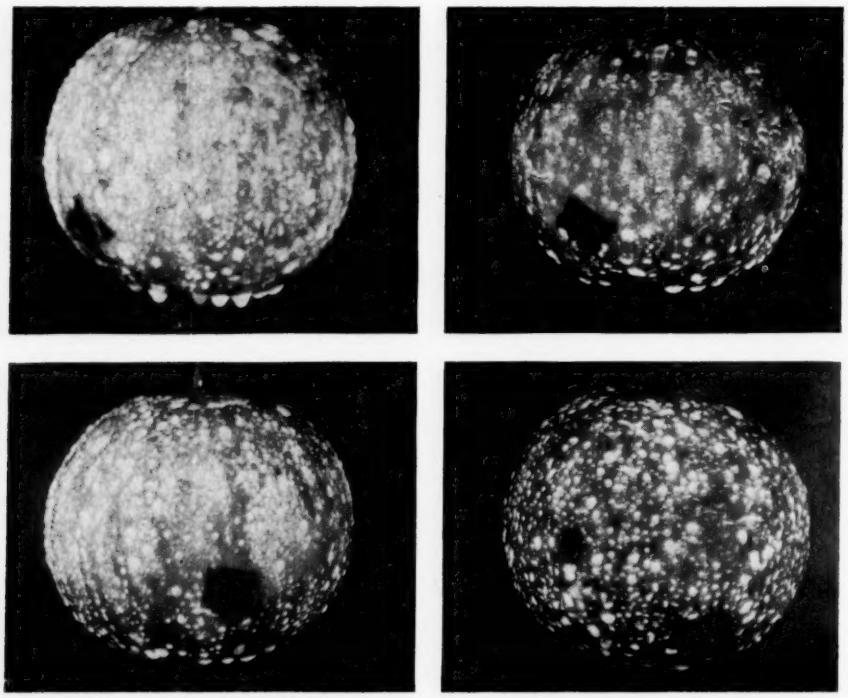

FIG. 1.—Deposit of oil A on mature grapefruit. Upper left—no surfactant. Upper right—0.5% Sponto 200. Lower left—0.25% 9D-207. Lower right—2.0% Sponto 200.

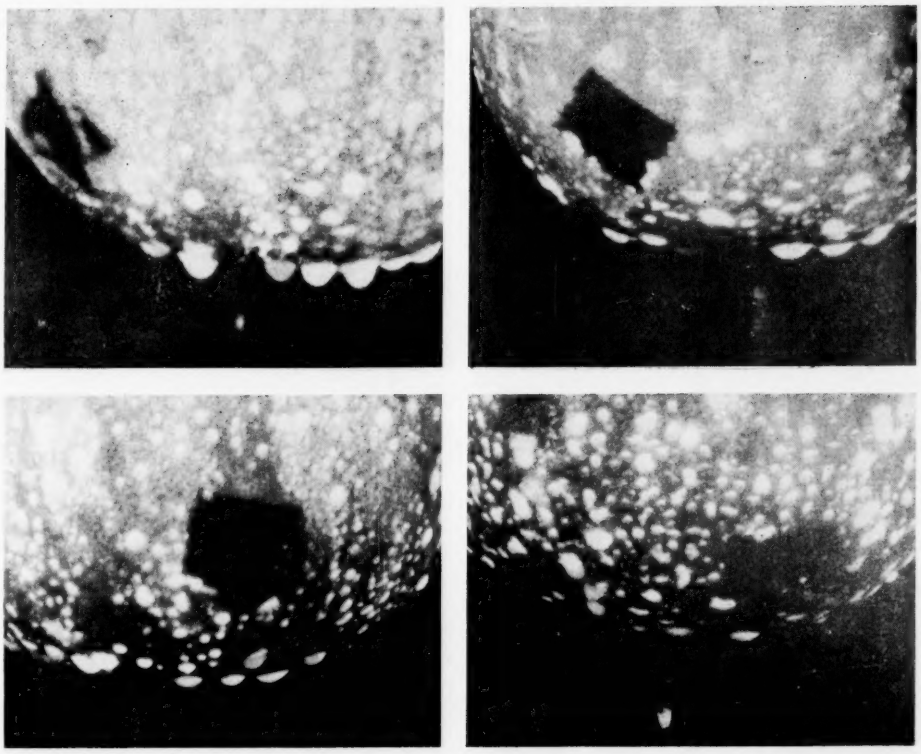

FIG. 2.—Deposit of oil A on mature grapefruit, enlarged. Upper left—no surfactant. Upper right—0.5% Sponto 200. Lower left—0.25% 9D-207. Lower right—2.0% Sponto 200.

with the absorbance of standard solutions prepared from the same dye and oil.

Selected, oil-soluble surfactants were added to various dyed oils to obtain desired surfactant concentrations. Spray mixtures were prepared by adding the emulsible oils to water. The percentage of actual oil in the various spray mixtures was held constant at 1.6% with the exception of a spray mixture containing only 0.1% which was used to compare the type of the deposit at two widely separated levels of amount of oil on green grapefruit.

Surface tension measurements were made on the various oil samples used, to determine if the addition of dye and/or surfactant affected this property. It was found that for a given oil, there was no change in surface tension by the addition of a surfactant, a dye, or a combination of both. The surface tensions of the formulations made from the naphthenic and paraffinic oils were approximately 36 and 35.5 dynes/cm., respectively.

Application of Spray.—The aqueous-oil-spray mixture was applied to the various plant parts according to a method described by Riehl *et al.* (1953) with modifications. A professional darkroom timer with automatic cut-off was used at 2-second intervals in order that the spray stream might operate during one revolution of the turntable. Mature grapefruit were sprayed in accordance with the routine given by Riehl *et al.* (1953) while immature green grapefruit picked from trees during July and August 1960 were sprayed during two revolutions of the turntable with the stem-stylar axis perpendicular to the spray stream. The upper end of the supplementary shaft of the turntable was drilled so the stainless steel spit could keep the fruit in this position for spraying. Leaves were held in the spray stream first with one side of the leaf toward the stream and then the other side. The leaves were then rotated in the spray stream for another 2 seconds. Green twigs and small branches (about $\frac{1}{2}$ inch and $1\frac{1}{4}$ to $1\frac{1}{2}$ inches in diameter) were sprayed in an upright position on the turntable during two revolutions. Twigs and small branches were drilled at the base to fit over the end of a paper clip which was wired to the turntable shaft.

Photographing Sprayed Citrus.—Positioning of various plant parts for photographing varied only slightly. The subject to film distance was 26.5 inches for leaves, twigs and small branches, and mature fruit; this distance was reduced to 16 inches for green fruit. A square patch of black plastic tape was placed on the various plant parts before spraying and was removed just prior to photographing. A Leica 111f camera with a 50-millimeter lens was used with a focaslide. A Kodak, Wratten K-2 filter was used in photographing leaves, green grapefruit and twigs while an X-1 filter was used with mature grapefruit. All pictures were made in a darkroom to exclude all stray light which might lead to a misinterpretation of

oil deposit. Short wave (Mineralight SL-2537), ultraviolet lamps were positioned 9 inches from the focusing point on the object. Two lamps were employed with mature fruit and leaves while three lamps were used with green grapefruit and green twigs and small branches. The distance of the lamps to the object was important as less fluorescence was noted at a 10-inch distance. Also, the amount of radiated light photographed was greater as the subject to film distance was decreased. Better results were found with super hypan film produced by Ansco with a daylight exposure index of 500.

Exposure times were varied while the f 16 lens opening was held constant. Exposure times for photographing were 11 minutes for the mature grapefruit in figure 1, 9 and 25 minutes for the green grapefruit in figure 3, 10 to 13 minutes for the leaves in figure 4, and 10 to 14 minutes for the plant parts in figure 5. A longer exposure time was found necessary where the intensity of fluorescence was weaker.

Oils and Surfactants Used.—Four oils were used in this investigation; selected properties of the oils are listed in table 2. Oil D is a proprietary emulsive California medium grade (Erb 1932, Marshall 1932) spray oil used in these trials as a reference spray oil; its formulation is not known. Oils A, B and C were prepared as a part of a program to define the desirable characteristics for petroleum spray oils for use on citrus in the Rio Grande Valley. Two oil-soluble nonionic surfactants (Sponto 200 and 9D-207)[5] of the type, alkyl aryl polyether alcohols and related compounds, were used to prepare emulsive type formulations of oils A, B, and C.

Table 2.—Physical properties of oils.

Inspections	Oils			
	Naphthenic Composition		Paraffinic Composition	
	A[a]	D	B[a]	C[a]
Viscosity at 100° F, SSU	73.3	68.5	80.2	105.0
Viscosity index	54	56	100	99
Unsulfonated residue, vol. %	94	92	92	90
Molecular weight (correlated)[b]	293	292	338	362
Distillation[c] temp., ° F. at				
5% distilled	602	590	666	724
50% distilled	674	664	727	757
95% distilled	736	795	801	809

[a] Prepared by Humble Oil and Refining Co.
[b] From correlation of Molecular Weight vs. Boiling Point and Gravity, J. B. Maxwell, Data Book on Hydrocarbons, D. Van Nostrand Co., Inc., New York.
[c] ASTM D-1160 10 mm. Vacuum Distillation corrected to 760 mm.

Discussion.—*Fruit.*—Drops of the spray tended to run downward on the surface of the grapefruit. In our procedure sprayed fruit were positioned with the axis from button to stylar ends in a vertical position and with the stylar end at the bottom. An accumulation of drops

[5] Sponto 200 manufactured by Retzloff Chemical Company, Houston, Texas, and 9D-207 manufactured by Rohm & Haas Company, Philadelphia, Pennsylvania.

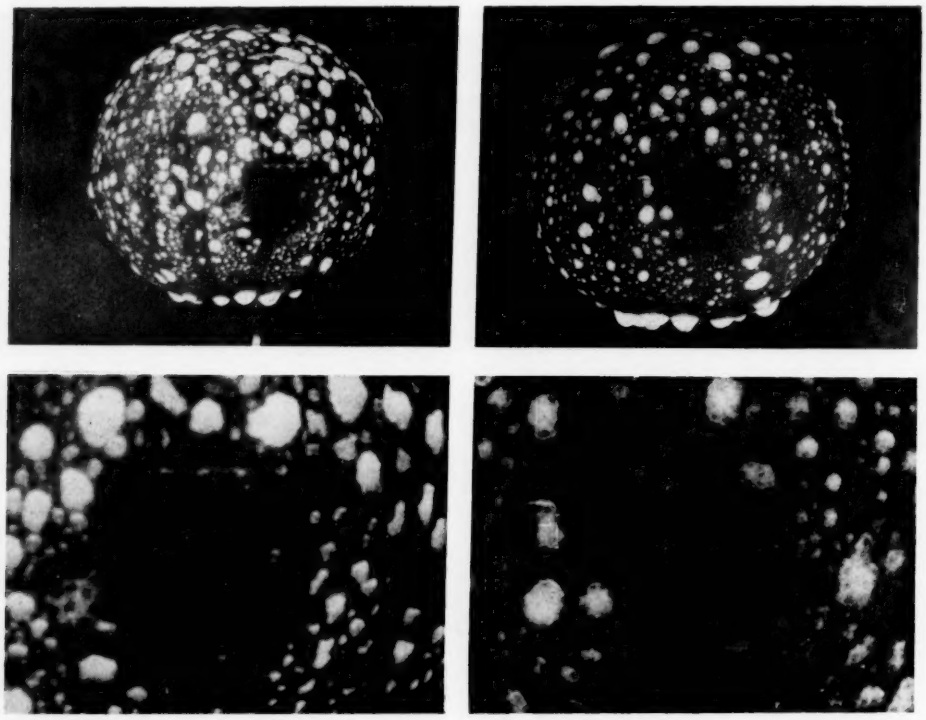

Fig. 3.—Deposit of oil A on young green grapefruit. Upper left—Sprayed with aqueous mixture of 1.6% oil (containing 0.5% Sponto 200). Upper right—Sprayed with aqueous mixture of 0.1% oil (containing 0.5% Sponto 200). Lower—enlargements of photos above.

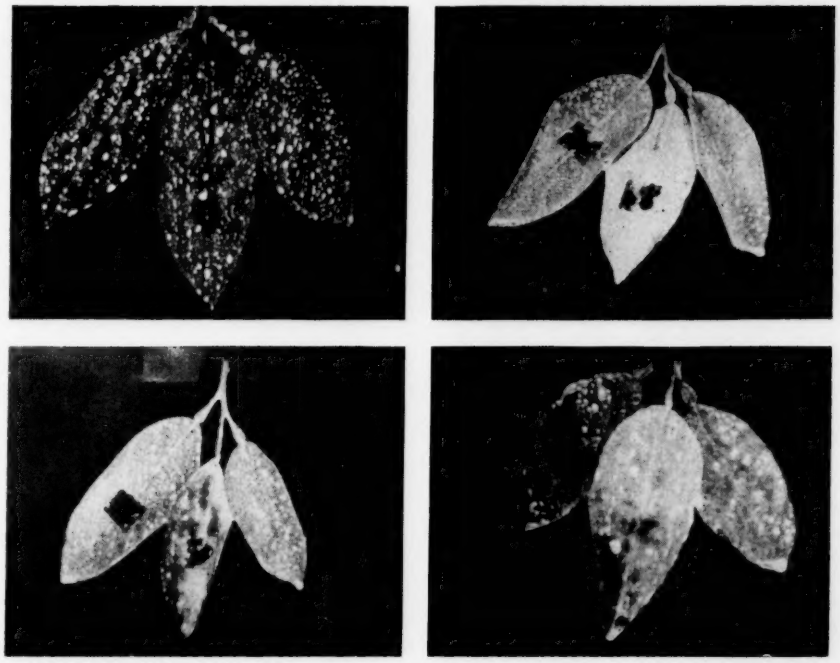

FIG. 4.—Deposit of petroleum oils on grapefruit leaves. Upper left—Oil D with unknown surfactant and 0.13% dye. Upper right—Oil C with 0.5% Sponto 200 and 0.16% dye. Lower left—Oil B with 0.5% Sponto 200 and 0.18% dye. Lower right—Oil A with 0.5% Sponto 200 and 0.17% dye.

was found around the button and stylar ends of the fruit, with larger drops observed on the stylar end.

The surface of the rind of grapefruit did not fluoresce under the ultraviolet light. Areas covered with the patch of plastic tape appear as black as the background in figures 1 and 2. Numerous large droplets are evident in the deposit on the fruit and these appear brighter than the area around them. It is likely that the amount of dyed oil is greater in the area of the drop and therefore is brighter under the ultraviolet light. However, the general surface area of the grapefruit between the drops is lighter than that of the area covered during spraying by the patch of plastic tape; this could only be due to fluorescence from the presence of oil containing Yellow C-4 Dye. The visual appearance of the fluorescence provides an indication of the type of oil deposit, the uniformity of distribution and a qualitative means of comparing the amount of oil present.

The upper left section of figures 1 and 2 shows the deposit from an aqueous mechanical mixture of oil A with no surfactant. A heavy deposit is indicated by the presence of very numerous large drops and by the overall brighter intensity of the fluorescence, particularly of the surface areas between the drops, indicating a thicker film of oil. On the other hand the lower right section of figures 1 and 2 shows a light deposit from a tighter spray mixture made with oil A containing 2.0% Sponto 200. The fluorescence shows that the overall film of oil on the lower right fruit is much thinner than that of the upper left fruit. On the lower right fruit the oil did not "break" out of the aqueous mixture and remain on the fruit surface in as heavy a deposit as on the other fruits of figures 1 and 2. The higher concentration of surfactant produced an emulsion with smaller drops of oil and stronger forces to keep the oil drops intact and suspended in the water phase. In this tighter mixture most of the oil was carried off of the fruit in the spray "runoff." Some portions of the general film of oil on the lower right fruit of figure 1 seem to be very thin and even discontinuous. Several comparisons of the larger drops of the upper left and lower right fruits of figures 1 and 2 are interesting and show that on the lower right fruit the drops are not as numerous and there is less oil associated with these drops because they have a smaller diameter and are flatter, *i.e.*, not as high or thick. The flatter profile is probably related to reduction of interfacial tension between the drop and the surface of the fruit as a net result of the surfactant content (Ebeling 1939).

In figure 1 the lower left fruit has a higher intensity of fluorescence than the upper right fruit. Comparisons in other determinations showed that the total amount of oil deposited was the same for spray mixtures made with oils containing 0.5% Sponto 200 and oils containing 0.25% 9D-207. In view of these results the dye content of the two oils was checked. It was found that the oil sprayed on the lower left fruit contained 0.19% dye while that sprayed on the upper right fruit contained only 0.09% dye. Therefore, it is to be expected that with the

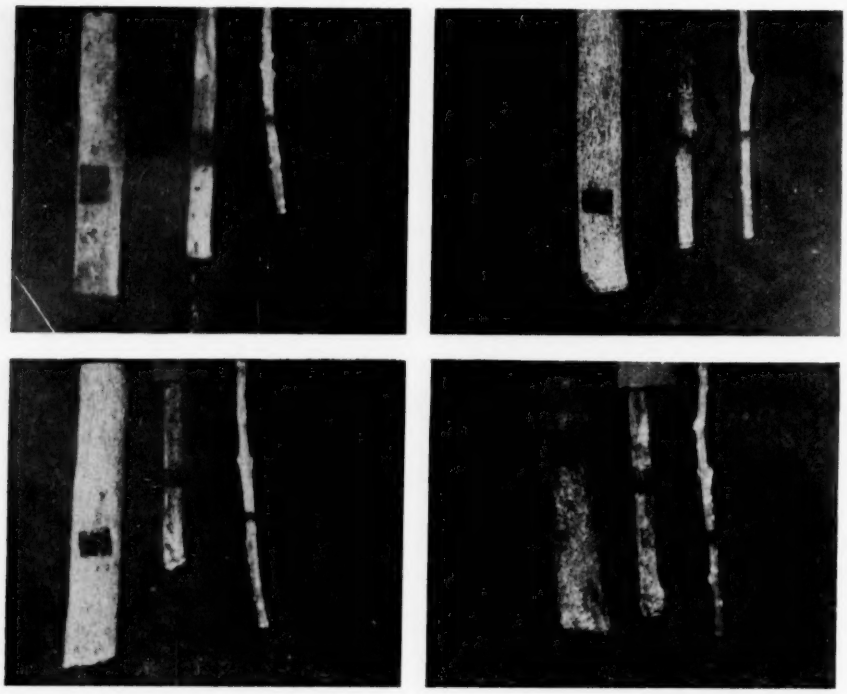

FIG. 5.—Deposit of various petroleum oils on grapefruit twigs and small branches. Upper left—Oil D with unknown surfactant and 0.13% dye. Upper right—Oil C with 0.5% Sponto 200 and 0.16% dye. Lower left—Oil B with 0.5% Sponto 200 and 0.18% dye. Lower right—Oil A with 0.5% Sponto 200 and 0.09% dye.

same oil deposit, the fruit sprayed with the oil having the greater dye content should give a more intense overall fluorescence. More drops appeared to occur on the sprayed fruit where 9D-207 surfactant was employed in the oil than where Sponto 200 was used. This was consistent where 0.5% Sponto 200 was compared with 0.25% 9D-207 and was also noted on fruit sprayed with oil containing 0.5% 9D-207. No conclusion is made at this time, however, that either one of the surfactants may be better for field use with various petroleum oils.

Observations of green fruit 24 hours after spraying indicated that the oil and dye had penetrated into the outer mesocarp of the peel. The fluorescence under ultraviolet light did not show the dye to have penetrated into the albedo. The albedo appeared purple under ultraviolet light. Amost 90% of the naringen of grapefruit was found in the albedo, rag and pulp, according to Anonymous (1956). When purified naringen was dissolved in approximately 1% citrus acid and observed under ultraviolet light, a purple fluorescence was observed. It is therefore probable that the purple fluorescence of the albedo in our observations was produced by naringen. A yellow fluorescence was observed in the flavedo of unsprayed grapefruit under 2537 Å ultraviolet light. When grapefruit peel was ruptured, the oil ejected on the fingers fluoresced yellow under ultraviolet light. A sample of purified grapefruit oil, when placed on filter paper, also fluoresced yellow under short-wave ultraviolet light. Anonymous (1956) reported limonene to be the principal constituent of the total citrus oil of grapefruit juice. It is therefore likely that the limonene is the principal cause of the yellow fluorescence observed in the flavedo of grapefruit. The fluorescene from the inner front of the oil penetration was quite distinct from the naturally occurring, fluorescent substance or substances; no effort was made to distinguish or identify them further.

Young green grapefruit picked from the tree and sprayed in early July or before showed greater penetration of oil and Yellow C-4 Dye into the outer mesocarp than fruits treated similarly in August. Only surface deposit on the outer mesocarp of mature grapefruit was noted; these mature fruit were of the 1959 crop and had been held in cold storage at 48° F. for 2 to 3 months. They were in good turgid condition and did not show any visible evidence of water loss.

Use of Yellow C-4 Dye to observe deposits of spray oil both immediately after application and just after the water had dried led to the opinion that the oil deposit consisted of discrete drops of oil which tended to remain separate on the fruit surface rather than to flow together. If this were true, decreasing the concentration of oil in the spray mixture should give a deposit with drops of the same size but less numerous per unit area of surface. Figure 3 shows a comparison of young green grapefruit sprayed with oil A at each of two concentrations: 1.6% on the left and 0.1% on the right. Both spray mixtures were prepared with oil which contained 0.5% Sponto 200. The interval of spray exposure was the same for both,

and spray runoff occurred on both fruits. The drops of both deposits seem to have about the same range in size and in number per unit area. However, the intensity of the fluorescence on the fruit sprayed with the 1.6% oil is much brighter than that treated with 0.1% oil even though the length of the time-exposure for photographing was three times longer for the latter. This indicated a much higher deposit of oil on the fruit sprayed with the 1.6% concentration. This evidence indicates that the size of the drops of the deposit is primarily a function of the water phase of the spray mixture, and that there is more oil per unit volume of water in the spray deposit left by the mixture containing 1.6% oil than in the one made with 0.1% oil. These observations suggest that "breaking" of aqueous-oil spray mixture at the plant surface may occur both as coalescence, within the water phase, of oil drops freed from the sheering stresses of the agitator, pump and nozzle but retarded by the surfactant present, and in part as release of free oil. This is in contrast to the general belief that most of the oil is released in free form immediately on contact of the spray with the plant surface. The information gained with the fluorescent technique was very revealing as to the dynamic role of the oil at the time of, and subsequent to, its deposit on the fruit surface. Further investigation of the subject certainly appears warranted.

Leaves.—Deposit of four different petroleum oils on grapefruit leaves is shown in figure 4. The upper left photo shows the deposit with oil D while that with oil A is shown in the lower right photo. The latter leaves were sprayed with oil A mixed with 0.5% Sponto 200 as were the leaves sprayed with the paraffinic oils B and C. The background fluorescence of the leaves sprayed with the naphthenic oils is not as intense as with the paraffinic oils. The background radiation of the deposit of the heavier paraffinic oil C, in the upper right photo, is more intense than that of the lighter paraffinic oil B, as shown in the lower left photo. The middle leaf in each photo shows the under surface of the leaf and has fewer large droplets than the leaves on either side which show the upper surface of the leaf. It is possible that the surfactants used in this study might function differently with the naphthenic and paraffinic oils as to their depositing patterns on grapefruit leaves. Also, the lower intensity of the fluorescence of the leaves sprayed with oil D is at least partly due to the lower dye content of this oil.

Grapefruit leaves were examined 24 hours after spraying to investigate the oil penetration pattern. The leaf surface was rubbed lightly with a cloth to remove the surface deposit of the dye. Fluorescence of the dye was found particularly around the midrib and larger veins. This was more easily observed when the leaves were allowed to dry and were then ground lightly with a pestle in a mortar to remove the areas of the leaf between the veins. Leaves held for 6 hours showed a large amount of the Yellow C-4 Dye deposited in crystalline form on the upper and lower surfaces of the leaves. Grapefruit leaves showed an accumulation of dye at the portion of the leaf in the downward position after spraying. Leaves that were torn showed an accumulation of dye at the torn edges.

Twigs.—Grapefruit twigs and small branches were sprayed to observe the relative deposit as shown in figure 5. Considerable runoff seemed to occur when twigs and small branches were held in the upright position. A heavier fluorescent glow appeared at the base of these plant parts when secured for photographing due to the downward flow of the oil-water mixture which was indicative of a heavier deposit accumulation in the downward areas. Larger droplets seemed to form only on the green twigs while a more uniform radiation appeared on the small branches than on the green twigs, except where bubbles occurred. The upper left and lower right photos in figure 5 show the deposit from two naphthenic oils, D and A, respectively, the latter containing 0.5% Sponto 200 surfactant. The upper right photo shows a green twig and two small branches sprayed with oil C while the plant parts in the lower left photo were sprayed with oil B. The paraffinic oils contained 0.5% Sponto 200. Penetration of the dye into the bark of green twigs initiated some speculation as to differential oil absorption into bark of various ages. When the bark of green twigs was peeled back, 1 day after spraying, the yellow dye was clearly shown to have penetrated well into such young bark. The dye, however, seemed to have deposited only on the surface of the bark of branches one-half inch in diameter and larger except in places where there was bark injury or bark irregularities from other causes. The evidence appeared contrary to the former belief (Ebeling 1950) that oil is rapidly absorbed into the bark of branches.

Grapefruit Tree.—A grapefruit tree about 12 feet high was sprayed with 1.6% of oil A, containing 0.5% Sponto 200 and 0.09% dye, to observe the relative oil deposit on various tree parts. Samples of leaves, fruit, and twigs taken from various parts of the tree and placed under ultraviolet light, provided good material for qualitative comparisons of oil deposit. Comparative observations were also made by placing the ultraviolet lamp in various parts of the tree at night. An attempt was made to photograph the fluorescence of the oil deposit on the tree at night under illumination with ultraviolet light. Four lamps did not give sufficient light to photograph the tree. The lamps were then placed in position to light up approximately one-fourth of the western half of the tree. Radiation from the dye under this intensity of ultraviolet light was not sufficient to produce a photographic image at exposure times of 10, 20, and 40 minutes with a lens opening of f 11. The dye content of the oil used to spray on the tree was only 0.09%. Had the concentration of the dye been greater, for example 0.19%, then the attempts to photograph the tree might have been successful.

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A New DDT-metabolizing Enzyme in the German Cockroach[1,2]

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ABSTRACT

A new microsomal enzyme system capable of converting DDT to a Kelthane-like product has been found in the German cockroach, *Blattella germanica* (L.). Kelthane® is 4,4′-dichloro-*alpha*-(trichloromethyl)-benzhydrol. The system requires oxygen, reduced triphosphopyridine nucleotide, magnesium ions and nicotinamide for activity. The enzyme(s) is also present in several strains of the house fly (*Musca domestica* L.) as well as in the American cockroach, *Periplaneta americana* (L.).

Several relatively polar DDT-derivatives are produced during the *in vivo* detoxication of DDT by the German cockroach, *Blattella germanica* (L.) (Hoskins *et al.* 1958). One of the metabolites detected—metabolite No. 4—has the same chromatographic behavior as Kelthane® (4,4′-dichloro-*alpha*-(trichloromethyl)-benzhydrol), a hydroxyl type derivative of DDT, reported to be produced in *Drosophila melanogaster* Meig. (Tsukamoto 1959). The conversion of DDT to Kelthane represents an oxidative process, with the peculiarity that only one atom of oxygen is incorporated into the DDT-molecule, resulting in the exchange of a hydrogen atom for a hydroxyl group. On the assumption that metabolite No. 4 of the German cockroach could correspond to Kelthane, the possibility that the biochemical mechanism involved in the formation of metabolite No. 4 could be of the mixed-function oxidase type (Mason 1957) was investigated. Mixed-function oxidases catalyze the transfer of one atom of oxygen to the substrate, the other atom undergoing reduction to water in the presence of a suitable electron donor. The latter, in some cases, has been shown to be reduced triphosphopyridine nucleotide (hereafter called TPNH) as for example, for a mammalian microsomal system mainly concerned with the hydroxylation of foreign aromatic compounds (Mitoma *et al.* 1956). We wish to report now the existence of an enzyme system in cell-free extracts of the German cockroach which in the presence of molecular oxygen, TPNH, nicotinamide, and magnesium ions catalyzes the conversion of DDT to metabolite No. 4. The enzyme(s) is located mainly in the microsomal fraction, and is also present in other insect species.

PROCEDURE.—Wild adult male German cockroaches were used throughout. Pre-chilled specimens were homogenized in 0.15 M KCl in a mortar with the aid of microglass beads at 2° C. and extracted for 60 min. at the same temperature. The resultant brei was filtered through cheesecloth to remove pieces of cuticle. To obtain subcellular fractions, the homogenate was centrifuged at 800×g for 10 min. to sediment the nuclei and cellular debris. The nuclear fraction (N) was washed twice with 0.15 M KCl and resuspended in an appropriate amount of the same medium. The supernatant fluid (fraction S) remaining after the centrifugation of the nuclei was used as a source of enzyme. For further fractionation, it was centrifuged at 9,000×g for 10 minutes to sediment the mitochondria. The pellet thus obtained (P_1) was washed twice with 0.15 M KCl before finally resuspending it in the same medium. The remaining supernatant fluid was then centrifuged at 100,000×g for 60 minutes to obtain a microsomal pellet (P_2). The remaining supernatant fluid (S_1) corresponds to the soluble fraction. All operations were conducted at 2° C.

Other species of insects used were: susceptible-SCR strain of house flies (*Musca domestica* L.) from Stauffer Chemical Company, Mountain View Research Laboratory and Parathion-resistant strain obtained from R. L. Metcalf, Citrus Experiment Station, Riverside, California. Lindane and DDT-resistant strains were selected in the laboratory from the SCR strain.

The enzyme assays were conducted in 10 ml. Erlenmeyer flasks at 37° C. with mechanical shaking. Unless otherwise stated, the atmosphere was air. Glass beads (200 mg.) were placed in the flasks, followed by 20 μmoles of C^{14}-DDT in acetone (specific activity, 12,000 c.p.m. per μmole). The solvent was removed under a stream of hot air. In addition to the DDT, the reaction mixture contained, unless otherwise stated, 80 μmoles of tris (hydroxymethyl)-aminomethane (Tris) buffer, pH 9.0; 60 μmoles of nicotinamide; 10 μmoles of $MgCl_2$; 2.4 μmoles of chemically reduced TPNH or 2.4 μmoles of TPN plus a TPNH-generating system consisting of 0.6 Kornberg units of crystalline yeast glucose-6-phosphate dehydrogenase, and 50 μmoles of glucose-6-phosphate; 1.0 ml. of enzyme preparation. The final volume was 2.0 ml. at a pH of 8.7, and the incubation time 120 minutes. The reaction was stopped by the addition of approximately 6 ml. of chloroform and the reaction mixtures were extracted with three additional portions of chloroform. A

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flask to which the enzyme was added after the addition of the chloroform was used as control. The combined extracts from each test were evaporated to dryness under a stream of hot air and the residue, dissolved in a minimum volume of acetone, spotted on Whatman No. 4 filter paper and chromatographically purified by the procedure of Menn *et al.* (1960) with acetonitrile. The purified extract from the strip was made to a volume of 1.0 ml. with acetone and an aliquot was taken for paper chromatography with β-methoxypropionitrile as stationary phase and *iso*octane saturated with β-methoxypropionitrile as mobile phase (Gordon 1960). After development, the chromatograms were scanned as previously described (Hoskins *et al.* 1958). The metabolite No. 4 was located and eluted with acetone and evaporated to dryness on a planchette, and the radioactivity measured by standard procedures using a Nuclear Chicago D-47 gas flow mylar window. All results are referred to the original fresh weight of the insect tissues. The ring labelled C^{14}-DDT was prepared in the laboratory by the condensation of chloral hydrate with C^{14} p-chlorobenzene in the presence of concentrated sulfuric acid and chlorosulfonic acid (Cook *et al.* 1947). The procedure utilized was based on the study of Mosher *et al.* (1946). Since the p-chlorobenzene was the limiting reagent, a 2 to 1 molar ratio of p-chlorobenzene to chloral hydrate was employed under micro scale conditions using the chlorosulfonic acid to achieve the necessary degree of catalysis for the condensation. The other reagents were of the highest purity commercially available. Sodium salts were used throughout.

RESULTS.—Table 1 shows some of the requirements for production of the Kelthane-like metabolite. TPNH and oxygen are absolute requirements for activity. When air is replaced by nitrogen, no production of the Kelthane-like metabolite is observed. Nicotinamide and especially magnesium enhance the activity. The fact that TPNH and not TPN is required is clearly demonstrated in table 1, since TPN is active only in the presence of a TPNH-generating system. The data of table 1 also show that neither diphosphopyridine nucleotide (DPN) nor DPNH is active in our system. It should be pointed out that G-6-P dehydrogenase alone or with G-6-P were also inactive in the absence of TPN. The possibility of a non-enzymic process can be discounted, since in the optimal system no activity was detected in the absence of cockroach enzyme or with boiled preparations (table 1). Reduced glutathione cannot replace TPNH. Cytochrome-c, methylene blue, flavine adenine dinucleotide (FAD) and adenosine triphosphate (ATP) were also inactive. The enzyme(s) was fairly unstable, all the activity being lost after storage at 2° C. for 48 to 72 hours. Optimal pH was found to be about pH 8.5–8.8, with Tris or phosphate buffer.

Table 1.—Production of Kelthane-like metabolite from DDT by cell-free extracts of the German cockroach.[a]

ADDITIONS	DDT CONVERTED (μMOLES/GM., FRESH TISSUE)
None	0.0
TPNH	0.83
TPNH-Mg^{++}	1.10
TPNH-Mg^{++}-Nicotinamide	1.16
TPN-Mg^{++}-Nicotinamide-TPNH-generating system	1.21
TPNH-generating system	0.0
TPN-Mg^{++}-Nicotinamide	0.0
DPN-Mg^{++}-Nicotinamide-DPNH-generating system	0.0
DPN-Mg^{++}-Nicotinamide	0.0
TPN-Mg^{++}-Nicotinamide-TPNH-generating system-boiled enzyme	0.0

[a] In addition to the previously described reaction mixture, all flasks contained 1.0 ml. of fraction S (780 mg. fresh weight of tissue). The DPNH generating system consisted of 200 μg crystalline liver 1-glutamic dehydrogenase and 50 μmoles of 1-glutamate. The above data represent three different experiments.

Table 2.—Intracellular distribution in the German cockroach of the system producing Kelthane-like metabolite.[a]

FRACTION[b]	DDT CONVERTED (μMOLES/GM.)	RELATIVE ACTIVITY (%)
Whole homogenate	1.58	100
Nuclei (N)	0.30	19
Mitochondria (P_1)	0.24	15
Microsomes (P_2)	0.71	45
Soluble (S_1)	0.0	0.0
Recovery	—	79

[a] *System*: 20 μmoles C^{14}-DDT; 80 μmoles "Tris" buffer, pH 9.0; 10 μmoles $MgCl_2$; 60 μmoles nicotinamide; 2.4 μmoles TPN; 0.6 Kornberg units G-6-P dehydrogenase; 50 μmoles G-6-P; 1.0 ml. enzyme preparation. Final volume, 2.0 ml.

[b] The fractions were derived from 3.5 grams of cockroaches. Incubated at 37° C. during 120 minutes.

Most of the activity was recovered in the microsomal fraction as the data of table 2 indicate. Slightly more than 20% of the original activity was lost during fractional centrifugation, suggesting that the conditions for obtaining the various subcellular fractions were not optimal.

Hoskins *et al.* (1958) have demonstrated the production of the Kelthane-like metabolite in susceptible house flies. Since this observation indicated that the enzyme system found in cockroaches might also be present in flies, several house fly strains as well as a wild strain of *Periplaneta americana* (L.) were studied in this respect. In all cases investigated, the enzyme was present (table 3). The highest level was found in a parathion-resistant strain which produced 3.36 μmoles of metabolite per gram. Susceptible, lindane-resistant and DDT-resistant flies had a very similar enzyme content, while the one found in American cockroaches was approximately of the same order of magnitude as in parathion-resistant flies.

DISCUSSION.—The similarity of the cockroach system to the one described in mammalian liver microsomes

Table 3.—Metabolite No. 4 *in vitro* production by cell-free extracts of various insect species.[a]

INSECT	DDT CONVERTED (μMOLES/GM.)[b]
Susceptible house flies	1.39
DDT-resistant house flies	1.44
Parathion-resistant house flies	3.36
Lindane-resistant house flies	1.48
American cockroach	3.20

[a] System as in table 2. Fraction S was used as a source of enzyme.

[b] These results have been confirmed in this laboratory by L. Kopelovitch, who has also found the enzyme in *Culex quinquefasciatus* mosquitoes.

(Mitoma *et al.* 1956) is striking. Both systems require oxygen, TPNH, and nicotinamide. However, while the liver system did not require any added cation, magnesium produced a slight stimulation of the cockroach enzyme(s), as in the case of Davison (1955). Whether hydrogen peroxide is involved in the conversion of DDT to the Kelthane-like product remains to be established. However, the fact that DPNH could not replace TPNH is an indication against the participation of H_2O_2. Furthermore, a hydrogen peroxide generating system cannot replace TPNH in the liver microsomal system (Gillette *et al.* 1957). Further evidence against a non-enzymic conversion of DDT to the Kelthane-like metabolite is the fact that the optimal pH of the non-enzymic model (Udenfriend *et al.* 1954) lies at the acid range, whereas our system had an optimal pH of 8.5–8.8.

Metabolite No. 4 produced *in vivo* by the German cockroach (Hoskins *et al.* 1958) and the *in vitro* product from DDT by our system have the same chromatographic characteristics as Kelthane (Tsukamoto 1959). Furthermore, the localization of the enzyme system in the microsomal fraction, as well as the requirements for TPNH, oxygen, nicotinamide and magnesium suggest that the *in vitro* DDT metabolite may very well be an hydroxylated product such as Kelthane. However, preliminary evidence based on the infrared spectrum of the *in vitro* metabolite indicates that although it is very similar to Kelthane the occurrence of additional ring hydroxylation may occur. Attempts to identify definitely the enzyme product are now being conducted, although proper purification of the metabolite appears to be extremely difficult due to the presence of interfering natural materials.

The existence of an oxidative enzyme system capable of degrading DDT *in vitro* appears to be quite unique, since the only known enzyme reaction involved in DDT degradation is dehydrochlorination, catalyzed by DDT-dehydrochlorinase (Lipke & Kearns 1959). The requirement of TPNH for DDT oxidation provides for the first time a link between normal metabolic reactions of the insect and DDT detoxication. One is tempted to correlate the availability of TPNH provided by the insect metabolic sequences, mainly the pentose phosphate pathway, with the rate of the *in vivo* DDT degradation by this system. DDT-dehydrochlorinase is activated by reduced glutathione (Lipke & Kearns 1959) which is provided essentially by glutathione reductase, a system requiring TPNH. Therefore, when the microsomal system and the soluble DDT-dehydrochlorinase are both present in an insect, there is the possibility of competition of both systems for TPNH. Whether DDT is detoxified via dehydrochlorination or via oxidation will then depend on the availability of TPNH, the affinity of both systems for TPNH, the cellular concentration and the turnover number of each system. This would explain why in some insects DDT is predominantly dehydrochlorinated, while in others polar metabolites are preferentially produced (Hoskins *et al.* 1958). It would also be appropriate to indicate here the striking similarity between this enzyme system and the system responsible for activation of phosphorothionates, found in rat liver microsomes (O'Brien 1959). Work on the purification and further characterization of the enzyme system is now being conducted in this laboratory.

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A Comparison of Some House Fly Bioassay Methods[1]

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Abstract

The house fly, *Musca domestica* L., has been used to compare some bioassay methods involving DDT as the standard insecticide, male, female, and a mixture of male and female flies, topical and film applications of DDT, and two bioassay designs. The results of these experiments were judged on the basis of assay validity, sensitivity, and efficiency. The most satisfactory procedure resulted from the use of 4-day-old female flies, topical application, and a 4×4 design composed of four 50-fly replicates for each of four graded doses of DDT. A computational protocol illustrates the use of the minimum logit chi-square method to obtain estimates of LD_{50} values and their reliability.

The relative potency of most chemicals that are toxic to arthropods and higher forms of animal life can be estimated rather precisely by insect bioassays. These techniques become of primary importance when chemical methods are unavailable or when neither the equipment nor personnel is available to conduct chemical analyses. Bioassays can also be used to provide an assuring check on chemical methods and sometimes provide unexpected dividends by revealing toxic metabolites which do not respond to the chemical procedures. Examples that illustrate these generalities are included in the reviews by Sun (1957) and Nagasawa (1959).

For more than a decade we have used the house fly, *Musca domestica* L., as a test organism because it is easy to rear and manipulate, and it responds quite well to most insecticides. Several different testing procedures have been employed (Dahm & Pankaskie 1949, Teotia & Dahm 1950, Stansbury & Dahm 1951, Dahm *et al.* 1959, and Gurland *et al.* 1960). Questions generated by these experiences prompted this study of some bioassay methods involving topical and film methods of toxicant administration and combinations of fly ages and sex, and experimental designs. These comparative experiments have led to the adoption of a standard bioassay procedure in our laboratories.

Experimental Procedures.—*Rearing and Selection of Flies.*—A laboratory strain of house flies, continuously reared from a 1948 NAIDM (National Association of Insecticide and Disinfectant Manufacturers) selection, was used for these experiments. Standardized fly rearing procedures were followed (Anonymous 1960), employing the Chemical Specialties Manufacturers' Association fly larval medium, an aqueous yeast suspension and nondiastatic malt solution, and water. Pupation occurred in a layer of sand which was added to the top of the larval medium about 2 days after eggs were added to the medium. The pupae were sifted from the sand and transferred to the floor of a screen wire adult emergence and holding cage which is approximately cubical with 29 cm. per dimension. About 2,000 pupae were placed in each cage. About 9 days elapsed between addition of eggs to the rearing medium and peak emergence of adults. The temperature in the rearing room was maintained at $28 \pm 2°$ C. and the relative humidity at $50 \pm 4\%$.

The flies were fed evaporated milk diluted with an equal volume of water. About 1% (w/v) of sucrose was added to the diluted milk. Food for the adults was prepared each day; the liquid was poured into glass bottles with a capacity of about 200 ml. and a top with a diameter of about 5 cm. The top was covered with four layers of cheesecloth held taut by a rubber band around the neck. The bottle was inverted over a piece of plastic screen with a mesh slightly smaller than that of the cheesecloth. The plastic screen and bottle of milk were placed on the wire screen top of a cage of flies. The wet circular area resulting from this arrangement provided both a feeding area for the flies and a place where the gravid females could lay their eggs. The latter were easily removed from the cheesecloth once a day when fresh milk was added. Because it is not necessary to open the cage for feeding and egg removal virtually no flies escape. This method of feeding flies and collecting eggs was brought to our attention by Dr. L. K. Cutkomp of the University of Minnesota.

Flies were selected at random and apportioned to groups for testing according to the requirements of the experiments described below. The age of the flies was counted from the day of maximum adult emergence. Groups of about 250 flies were drawn by an air current from a holding cage to cylindrical cardboard cartons. The flies were anesthetized with carbon dioxide and either males, females, or mixtures of both sexes selected according to the needs of the experiment. The sexual dimorphism of the eyes and posterior abdominal segments was used to distinguish between males and females (West 1951). The flies were counted into groups of 50 and then transferred to 1-pint cylindrical cardboard cartons. Sufficient time was allowed to determine whether all the flies in each group recovered from the effects of anesthetic and handling. Only occasionally did a fly have to be removed and a replacement added because of injuries caused by these manipulations. In addition to the flies used for testing purposes, another group of 50 flies was similarly selected and immediately weighed to obtain an estimate of the average body weight of the flies from each culture used.

Bioassay Methods.—A sample of *p,p'*—DDT (m.p. 108–9° C.) was used as the standard insecticide. Experiments were designed to compare the bioassay efficiency of topical applications of DDT to the flies with continuous exposure of the flies to a film of DDT on glass. The primary experimental method consisted of four 50-fly replicates for each of four graded doses of DDT. This type of bioassay was designated as a 4×4 experiment. For comparison, bioassays were also performed using three repli-

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[2] The first and last authors are from the Department of Zoology and Entomology; the other authors are from the Department of Statistics. The careful and dependable help of Guy Stockdale in carrying out these experiments is gratefully acknowledged by the authors.

cates of 50 flies each and five graded doses of DDT (3×5 experiments).

In the topical application experiments acetone solutions of DDT were applied to the mesonotum of flies with either a hand-operated or automatic microapplicator (Hamilton & Dahm 1960). Both devices consisted essentially of a 0.25-ml. syringe and a number 27 hypodermic needle; the syringe plunger was moved by turning the barrel of a micrometer caliper. Approximately 1 μl. of an acetone solution of DDT was applied per fly. A standard solution of DDT in glass-distilled acetone was used to prepare appropriate test solutions. The range of doses and intervals between doses were varied according to the response obtained from flies of different ages and sex and the experimental design used. In general, the doses ranged from 0.1 to 0.5 μg. of DDT per fly and the dose intervals were usually 0.08 and 0.06 μg. DDT for the 4×4 and 3×5 experiments, respectively. Each group of 50 flies was lightly anesthetized with carbon dioxide and the flies treated at the rate of about 10 flies per minute, including time spent changing or replenishing solutions in the syringe. Each treatment series included one or two groups of flies which were treated with acetone only, usually one group of flies at the beginning and another group at the end of the DDT treatments. Low mortality among these flies indicated the absence of toxic amounts of DDT in the acetone-rinsed syringe and that the acetone had little, if any, toxic effect. Another group of 50 flies was carried through the experimental procedures, except for treatment, to indicate the natural mortality under these circumstances. Generally only 1 or 2 flies out of 50 were dead in this control group of flies after 24 hours. The treated and still anesthetized flies were gently shaken into a 1-quart, wide mouth, glass Mason jar. A paper cup containing Cellucotton saturated with the milk solution used to feed the adult flies was placed in the horizontally held jar. A screen wire lid was added and the jar placed in a horizontal position on a shelf in a room where the temperature was about the same as that in the rearing room.

The film method of continuously exposing flies to DDT consisted of adding graded doses of DDT dissolved in acetone or benzene to 1-quart, wide mouth, glass Mason jars. The doses of DDT ranged between 10 and 80 μg. per jar; the dose intervals varied from 10 to 15 μg. depending upon the sex and age of the flies and the experimental design. The equivalent of 0.2 mg. of corn oil, dissolved in the solvent, also was added to each jar. The insecticide and corn oil were dispersed over the interior surface of the jar by rotating the horizontally held jar as the solvent evaporated. Groups of 50 flies were selected as previously described. The flies were transferred to the test jars, milk was added to each jar, and the jars were closed with screen wire lids. Both 4×4 and 3×5 experimental designs were used. The flies exposed themselves to the DDT as they moved at random about the jar. Groups of flies were also added to jars containing only corn oil, and at least one group of flies was added to a jar containing neither DDT nor corn oil to indicate the control mortality. Usually none or at most one or two flies died in this jar.

In all the experiments mortality estimates, including both dead and moribund flies, were made at the end of 24 hours. There were only a few moribund flies after 24 hours; the maximum numbers of moribund flies occurred about 10 hours after exposure to DDT. The actual number of flies in each jar was obtained after killing the living flies with heat and counting the total number of flies in the jar. This number was used in calculating the per cent mortality. Between assays the jars and their lids were decontaminated by washing and rinsing in acetone.

Statistical Analysis.—The minimum logit chi-square (χ^2) method (Berkson 1953) was used in analyzing the data because the calculations by this method are simpler than by the method of probits using maximum likelihood. The minimum normit chi-square method (Berkson 1955) could also have been used to provide a relatively simple method of computation; however, the difference in using the normit and logit transformations is inconsequential. In order to illustrate the use of the logistic function in analyzing bioassay data an actual computation is presented in table 1. To explain the symbols used in the table, a few brief definitions are necessary.

If P_i represents the probability of a fly being killed at the ith dosage x_i (log dose) and a logistic distribution of tolerance dosages is assumed, then

$$P_i = \frac{1}{1 + e^{-(\alpha+\beta x_i)}} \tag{1}$$

the logit transform (this terminology is due to Berkson) is

$$L_i = \text{logit } P_i = \ln \frac{P_i}{1 - P_i} = \alpha + \beta x_i \tag{2}$$

so that if logit P_i is plotted against x_i the points will fall on a straight line with α as intercept and β as slope.

Suppose k groups consisting of $n_1, n_2, \cdots, n_k$ house flies are exposed to dosages $x_1, x_2, \cdots, x_k$ respectively, and r_i flies respond (dead plus moribund) at the ith dosage.

Write the observed proportion affected as

$$p_i = \frac{r_i}{n_i}$$

The empirical logit of p_i is

$$l_i = \text{logit } p_i = \ln \frac{p_i}{1 - p_i}$$

Berkson's logit χ^2 to be used for estimating the parameters α, β has the form

$$\chi^2 \text{ (logit)} = \sum_{i=1}^{k} n_i w_i (l_i - \alpha - \beta x_i)^2 \tag{3}$$

where the weight $w_i = p_i(1-p_i)$.

Estimates of the parameters α, β are obtained by minimizing the expression in (1). The estimates a, b, so obtained are computed in table 1.

From equation (2), log LD_{50} is given by

$$-\frac{\alpha}{\beta}, \text{ and it is estimated by } -\frac{a}{b}$$

as shown in table 1.

The analysis of variance summarized in table 2 is based on the results shown in table 1. The sum of squares for the deviations from regression and for between repli-

Table 1.—Computation for bioassay data from a 4×4 experiment; approximately 1μl. of acetone solutions of DDT were applied to 4-day-old female house flies.

Dose (z) (μG./FLY)	Log Dose (x)	Flies per Replicate (n)	% Dead +Moribund (p)	Corrected % Dead +Moribund[a] (p')	nw[b]	nwx	nwl[c]
0.30	−0.52288	50	16	15.2	6.445	− 3.36996	−11.07896
		50	16	15.2	6.445	− 3.36996	−11.07896
		50	10	9.1	4.135	− 2.16211	− 9.51666
		50	8	7.1	3.300	− 1.72550	− 8.48572
					20.325	−10.62753	−40.16030
0.38	−0.42022	50	22	21.2	8.355	− 3.51094	−10.96936
		49	33	32.3	10.716	− 4.50320	− 7.93028
		50	26	25.3	9.450	− 3.97108	−10.23133
		50	30	29.3	10.360	− 4.35348	− 9.12571
					38.881	−16.33870	−38.25668
0.46	−0.33724	50	40	39.4	11.940	− 4.02665	− 5.14053
		50	44	43.4	12.280	− 4.14131	− 3.26095
		50	46	45.5	12.400	− 4.18178	− 2.23808
		50	54	53.5	12.440	− 4.19527	1.74446
					49.060	−16.54501	− 8.89510
0.54	−0.26761	46	70	69.7	9.715	− 2.59988	8.09325
		50	74	73.7	9.690	− 2.59314	9.98487
		50	76	75.8	9.170	− 2.45398	10.46985
		50	58	57.6	12.210	− 3.26752	3.74078
					40.785	−10.91452	32.28875
Control		100	1				

[a] $$p' = \frac{M_o - M_c}{100 - M_c},$$

where p' = corrected % dead + moribund, M_o = the observed % mortality, M_c = the control % mortality. When $M_o \leq M_c$, use the relation:

$$\left(\frac{1}{2n}\right) \cdot 100;$$

see Berkson (1953), page 584, for a discussion of this empirical rule of dealing with 0% and 100% mortalities.

[b] w = logistic weights from table 3, Berkson (1953).

[c] l = logits from table 1, Berkson (1953).

$\Sigma nw = 149.051$ $\Sigma nwx = -54.42576$ $\Sigma nwl = -55.02333$

$$\bar{x} = \frac{\Sigma nwx}{\Sigma nw} = -0.36515 \qquad \bar{l} = \frac{\Sigma nwl}{\Sigma nw} = -0.36916$$

$\Sigma nwx^2 = 20.92325$ $\Sigma nwxl = 31.48423$ $\Sigma nwl^2 = 155.05695$

$\frac{(\Sigma nwx)^2}{\Sigma nw} = 19.87349$ $\frac{(\Sigma nwx)(\Sigma nwl)}{\Sigma nw} = 20.09169$ $\frac{(\Sigma nwl)^2}{\Sigma nw} = 20.31229$

$\Sigma nw(x - \bar{x})^2 = \text{Diff.} = 1.04976$ $\Sigma nw(x - \bar{x})(l - \bar{l}) = \text{Diff.} = 11.34254$ $\Sigma nw(l - \bar{l})^2 = \text{Diff.} = 134.74466$

$$b = \frac{\Sigma nw(x - \bar{x})(l - \bar{l})}{\Sigma nw(x - \bar{x})^2} = 10.80489 \qquad a = \bar{l} - b\bar{x} = 3.57625$$

Regression equation: $a + bx = 3.58 + 10.80x$

log LD_{50} estimate: $m = -\frac{a}{b} = -0.33098$ Estimated LD_{50}: antilog $m = 0.467$ (μg./fly)

$$\text{var}(b) = \frac{1}{\Sigma nw(x - \bar{x})^2} = 0.95260; \quad \sqrt{\text{var}(b)} = 0.976 \qquad \text{var}(m) = \frac{1}{b^2}\left[\frac{1}{\Sigma nw} + \frac{(m - \bar{x})^2}{\Sigma nw(x - \bar{x})^2}\right] = 0.0006699; \quad \sqrt{\text{var}(m)} = 0.0259$$

95% confidence interval for b: $b \pm 1.96\sqrt{\text{var}(b)} = 10.80 \pm 1.91$

95% confidence interval for m: $m \pm 1.96\sqrt{\text{var}(m)} = -0.33098 \pm 0.05076$

95% confidence interval for LD_{50}: $0.415 < LD_{50} < 0.524$ (μg./fly)

S.S. (Sum of squares) Between doses $= \frac{(-40.16030)^2}{20.325} + \frac{(-38.25668)^2}{38.881} + \frac{(-8.89510)^2}{49.060} + \frac{(32.28875)^2}{40.785} - 20.31229 = 123.85828$

S.S. Regression $= \frac{(11.34254)^2}{1.04976} = 122.55488$

S.S. Deviation from regression $= 123.85828 - 122.55488 = 1.30340$

S.S. Between replicates/dose $= 134.74466 - 123.85828 = 10.88638$

Table 2.—Analysis of variance for the data of table 1.[a]

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE
Regression	1	122.55488	
Deviations from regression	2	1.30340	0.65170
Between doses	3	123.85828	
Between replicates within dose	12	10.88638	0.90720
Total	15	134.74466	

[a] Finney 1952, pages 163–5.

cates within dose are of particular importance. The former value, 1.30340, is a $\chi^2_{(2)}$ (chi-square with 2 degrees of freedom) which measures departures from linearity. The latter value, 10.88638, is a $\chi^2_{(12)}$ which provides a test of heterogeneity of the response of the flies for different batches at the same dose level. Both of these χ^2 values are nonsignificant when tested against tabulated values. It should be noted that the sum of squares for deviations from regression and for between replicates within dose are added to give the residual value which is identical to $\chi^2_{(14)}$. This value provides an ordinary test of linearity. The significant χ^2 values for "deviations from linearity" are not solely attributable to the real systematic deviations of the transformed response of the flies from the regression line (Finney 1952). Heterogeneity between replicates at the same dose may increase both sums of squares.

To indicate the relative precision and sensitivity of the two bioassay methods, weighted means of estimated slopes of the dosage response regression equations were computed. These are shown in table 3 for both the topical and film methods. The weighted means of LD_{50} estimates for both methods are also shown in table 3. These are expressed as μg. DDT/fly for the topical method and μg. DDT/jar for the film method. In computing the weighted means of estimated slopes and the weighted means of the LD_{50} estimates, the reciprocals of the variances of the estimated LD_{50} values and slopes respectively were used as weighting factors (Burn *et al.* 1950).

RESULTS AND DISCUSSION.—The bioassays performed with 4-day-old house flies are summarized in table 3 wherein topical and film methods of exposing the flies to DDT are compared on the basis of (i) the number of experiments in which the computed chi-square values were significant at least at the 5% level, (ii) the weighted mean of estimated LD_{50} values, and (iii) the weighted mean of estimated slopes. The topical method resulted in only 8 experiments of 40 as compared with 29 of 35 for the film method for which the computed chi-square values are significant. The criteria used to establish validity have been illustrated in the computation example listed in tables 1 and 2. Although it is not possible to estimate the quantity of DDT picked up per fly by the film method, if one assumes that the 50 flies per jar could pick up all the DDT, then one obtains theoretical values of 1.27, 1.77, 0.48, 0.42, 0.84, 0.87 μg. DDT/fly for the six film assay series listed in table 3. If these numbers are divided by the corresponding LD_{50} values for the topical application experiments it appears that about two to five times more DDT was required in the films to produce the responses obtained by topical application of the insecticide. This greater precision of the topical method is especially important in estimating low residues. The greater sensitivity of the topical method is also evident from a comparison of the slope values; those of the topical bioassays are about twice as steep as those for the film method. Thus in these experiments the topical bioassays provided greater precision and sensitivity than those performed using the film method.

The average weight of fifty 4-day-old flies selected with each topical assay series summarized in table 3 was used to compute the following LD_{50} values: 16.9, 11.4, and 15.8 μg. DDT/g. fly body weight for the assays with female, male, and mixed male and female flies, respectively. The mixture of male and female flies contained, on the average, about 40% males. Although the lower LD_{50} values for the assays with male and mixed male and female flies suggest that the use of male flies would increase the sensitivity of the assays, the variation in numbers of males in the cultures actually made it difficult on some days to obtain enough 4-day-old male flies for an assay series. The superior response of 4-day-old female flies to handling, selection, and the doses of DDT has caused us to use only 4-day-old female flies for analytical bioassays. It takes very little extra time to select only female flies by the counting procedures described above.

The results of the significance tests applied to the

Table 3.—A comparison of bioassays with 4-day-old house flies; the LD_{50} and slope values are weighted as described in the text.

SEX	BIOASSAY DESIGN	TOPICAL (i) Bioassay Experiments[a]	TOPICAL (ii) Mean LD_{50} (μg./fly)	TOPICAL (iii) Mean Slope	FILM (i) Bioassay Experiments[a]	FILM (ii) Mean LD_{50} (μg./jar)	FILM (iii) Mean Slope
Female	4×4	15 (3)	0.253	7.89	7 (6)	63.29	3.87
	3×5	5 (0)	0.448	10.65	4 (4)	88.47	4.05
Male	4×4	5 (3)	0.224	7.49	7 (7)	23.91	4.46
	3×5	5 (2)	0.209	7.79	7 (6)	21.26	3.02
Males and females, mixed	4×4	5 (0)	0.399	6.81	6 (4)	42.17	3.56
	3×5	5 (0)	0.340	7.29	4 (2)	43.55	3.43

[a] The values in parentheses indicate the number of experiments in which deviation from the regression line and heterogeneity of the response of the flies were significantly large at the 5% level.

4×4 experiments suggest, in the light of the many non-significant chi-square values for variation between replicates, that an assay consisting of four graded doses for the standard with two groups of fifty 4-day-old flies per dose would be quite adequate. Although a similar modification of the 3×5 design would also be reasonable, we prefer the 4×4 design for the following reasons. Firstly, if five doses of the toxicant are distributed uniformly over the same range as four doses, with the expectancy that both series of doses will cause mortalities ranging from about 15% to 85%, the closer the doses are to each other the more often a group of flies will have a higher mortality with a lower dose than another group of flies at the next higher dose. The frequency of this reversal in response increased when the interval between doses was 0.05 μg. DDT/fly or less in the topical application experiments. Secondly, the economy resulting from the use of four instead of five doses, with two replicates per dose, is substantial.

The bioassays summarized in table 3 were performed over a period of several months and not in any particular sequence. The variations in LD_{50} and slope values between assay designs emphasize the importance of using parallel line assays and estimating the relative potency of the test chemical in terms of the ratio of its LD_{50} value to that of the standard assay. Although the response of the flies may vary somewhat from one assay series to another, the relative potency of a given test preparation should be quite similar from bioassay to bioassay as long as proper conditions of linearity and parallelism prevail.

Comparisons similar to those summarized in table 3 were also made using 2-day-old flies. The principal result of this change was to obtain slightly lower values for the LD_{50} estimates. Flies from two other cultures, a light-eyed mutant strain developed from the 1948 NAIDM selection and a wild strain developed from flies collected in the vicinity of Ames, Iowa, during the summer of 1959, were also used for comparative purposes. The mutant flies responded in essentially the same way as the flies from the 1948 NAIDM selection. Flies from the wild strain were about two times as resistant to the DDT as the flies from the 1948 NAIDM selection but the regression lines had approximately the same slopes as the lines obtained with the more susceptible flies.

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Lodgepole Needle Miner Parasites: Biological Control and Insecticides[1]

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ABSTRACT

A malathion-oil spray program against the lodgepole needle miner (*Recurvaria milleri* Busck) at Yosemite National Park in 1959 was detrimental to the defoliator's principal primary parasites. During the control operation, information was gained through the use of visual observation, catch cloths placed beneath treated and untreated trees and analyses of effectual residue on contaminated foliage in small cages. From data obtained during the investigation coupled with available knowledge of pest-parasite ecology, it is concluded that innovations in future needle miner control programs might provide for greater conservation of natural enemies.

The lodgepole needle miner, *Recurvaria milleri* Busck[3] (Lepidoptera: Gelechiidae) has caused various degrees of defoliation on approximately 60,000 acres of lodgepole

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[2] Lodgepole needle miner parasite investigations by the University of California, Department of Biological Control are in collaboration with the Pacific Southwest Forest and Range Experiment Station, U. S. Forest Service and Yosemite Park.

[3] North American lodgepole needle miners formerly of the genus *Recurvaria* are now placed in the genus *Eragora* (Freeman, T. N. 1960. Needle-mining Lepidoptera of pine in North America. Canadian Ent. **92** (suppl. 16): 1-51). *Eragora milleri* (Busck) and *Eragora starki* (Free.) = *Recurvaria milleri* Busck and *Recurvaria starki* Free. of previous authors. Freeman's taxonomic change occurred too late to allow for its incorporation into the text of this publication.

pine, *Pinus contorta* Dougl., in Yosemite National Park, California, on the headwaters of the Tuolumne and the Merced Rivers (Anonymous 1960). Now in its 14th year, the outbreak continues to increase in area and severity, leaving thousands of dead and weakened trees in its wake. Many of these weakened trees are killed by secondary attacks of the mountain pine beetle, *Dendroctonus monticolae* Hopk., which also approaches outbreak proportions in localized regions of recent needle miner defoliation.

By an agreement between the United States Forest Service and the National Park Service, entomologists of the Pacific Southwest Forest and Range Experiment Station have been investigating the lodgepole needle miner at Tuolumne Meadows since 1954. In 1958, the Department of Biological Control of the University of California, in collaboration with the Forest Service, initiated more extensive research concerning its natural enemy complex.

THE PROBLEM.—In the Tuolumne Meadows area the current outbreak is the third since 1910 (Struble & Bedard 1958). The causes for these periodic needle miner increases and declines in California are not known. However, it is postulated that a closely allied species, *Recurvaria starki* Free,[3] in Canada, was caused to increase to outbreak proportions by climatic release (Stark 1959b). That outbreak continued in the Canadian Rocky Mountains for 10 years during a period of favorable weather and was subsequently terminated by sustained adverse winter temperatures of −30° F. to −40° F. (Stark 1954, 1959a, Henson *et al.* 1954). Stark (1959a) considered that natural enemies did not figure prominently in the initiation, course and termination of the recent *R. starki* outbreak.

The situation at Yosemite differs from that in Canada, principally in three ways. First, there is a different pest species involved, although *R. milleri* and *R. starki* superficially appear quite similar in habit. Second, there is a consistently more favorable climate at Tuolumne Meadows. Third, lodgepole pine in the Yosemite outbreak area is considered to be both successional to red fir and, in many localities, a climax species (the lodgepole-hemlock faciation of Oosting, 1948). Lodgepole pine at the sites of the Canadian outbreak is considered a fire sub-climax type.

It is quite possible that favorable climatic conditions fostered the release of *R. milleri* in California though supporting data to that effect have not been obtained. On the other hand, general knowledge of the infested area discourages prognosis for termination of the present outbreak by sustained low winter temperatures such as were involved in the Canadian situation.

Under the edaphic and climatic conditions governing the distribution of lodgepole pine and associated species at Tuolumne Meadows, a relatively stable biotic community might be expected. Frequent and prolonged superabundances of one of the component species of this community would seem unusual. We cannot yet exclude the possibility that the work of the defoliator and the mountain pine beetle might contribute to a natural periodic rejuvenation of lodgepole stands by the removal of older mature trees. Yet, it is difficult to rationalize the superfluous nature of the needle miner in this process and, as well, its violence and frequency.

Whether or not *Recurvaria* outbreaks at Yosemite are natural and normal ecological processes, National Park administrators judge them detrimental to the preservation of specific situations considered favorable to the Park. Their prevention and control, therefore, have become urgent local problems. Barring the possibility that climate alone might determine the fate of these outbreaks, the possibilities of control by biotic factors should be considered. A better knowledge of the lodgepole needle miner and its natural enemies may disclose the cause of these periodic infestations and more effective means for combating them.

THE PEST AND ITS NATURAL ENEMIES.—*Recurvaria milleri* and closely related species on lodgepole pine normally require 2 years to complete their life cycles. The needle miner in California is rather unusual because each generation is apparently discrete; overlapping broods have never been reported here. At Tuolumne Meadows each generation of *Recurvaria* overwinters twice, once principally in the first instar and once principally in the fifth instar (Struble 1960). Moths normally emerge over a period between early July and early August. Females may survive approximately 3 weeks, whereas males are short-lived and often die shortly after mating (Morgan 1956). Oviposition reaches a peak in early August and is followed by eclosion in late August and September. The young larvae then enter the needles where they remain during the ensuing winter. At Tuolumne Meadows moths appear only in the odd numbered years, *e.g.*, 1957, 1959 (Patterson 1921, Struble 1958a).

As is characteristic of many other indigenous insects in essentially unaltered habitats, the needle miner has a complex association of natural enemies involving many species. Yet, of these only the hymenopterous parasites have caused appreciable host mortality. The complexity of the relationships between *Recurvaria* and its parasites is compounded by the host's discrete 2-year life cycle. The aspects of these host-parasite relationships as they affect outbreak conditions are currently under investigation. This work, and that of predecessors (Patterson 1921, Yuill 1939, Struble & Bedard 1958, Struble & Martignoni 1959), have associated 24 parasite species with the needle miner at Yosemite but there is strong indication that this list will be increased to 30 or more. Of these, 16 are known only as primary parasites. Two species appear to be obligatory hyperparasites, while six act as primary as well as secondary parasites.

Of the known primary parasites it would appear, at this time, that at least five species either cause significant host mortality or have a potential capacity to do so. These are as follows:

1. *Copidosoma deceptor* Miller (Encyrtidae) an essentially specific, host synchronized, egg-larval parasite.
2. *Apanteles californicus* Mues. (Braconidae) an essentially specific, host synchronized, internal larval parasite.
3. *Dicladocerus* sp. (Eulophidae) a nonspecific, asynchronous, external larval parasite. *Sympiesis stigmatipennis* Gir. is similar in habit but currently less abundant.
4. *Zagrammosoma americanum* Gir. (Eulophidae) a nonspecific, asynchronous, usually solitary, external parasite. Because of its tendency toward facultative hyperparasitism the beneficial nature of this species might, under unique circumstances, be jeopardized.

5. *Aethecerus pinifolii* Mason (Ichneumonidae) a non-specific, asynchronous, solitary, internal pupal parasite.

Copidosoma deceptor and *Apanteles californicus*, being relatively host specific and synchronized, have the capacity to bring about effective reduction of *Recurvaria*. *Copidosoma* is comparatively efficient despite certain limitations of its niche. *Apanteles* is far less effective than it might be at this time. The eulophids and ichneumonids bring about significant host mortality rather consistently. However, the presence of effective numbers of these parasites must be regarded as less reliable than that of the synchronized species.

Hyperparasitism shall be mentioned only briefly because information concerning this phase of the study is still preliminary in nature. Secondary parasites have been abundant during recent flight years and appear capable of limiting the effectiveness of the needle miner's primary parasites. Observations suggest that hyperparasitism, particularly by *Amblymerus* sp., is in part responsible for the reduced efficiency of *Apanteles* at this stage of the outbreak.

The species most commonly recovered as hyperparasites are:

1. *Amblymerus* sp. (Pteromalidae) females have been recovered from *Apanteles californicus*, parasites of *Recurvaria* pupae (probably *Aethecerus pinifolii*) and rarely as primary parasites from *Recurvaria* larvae. Males are common parasites of *Copidosoma deceptor*.
2. *Tetrastichus* (prob.) *coerulescens* Ash. (Eulophidae) have been recovered from pupae of primary eulophids and from *Recurvaria* larvae mummified by *Copidosoma deceptor*.
3. *Tetrastichus* sp. (Eulophidae) have been recovered from *Copidosoma deceptor*.
4. *Chrysocharis* sp. (Eulophidae) females have been recovered from *Aethecerus pinifolii* and *Apanteles californicus* but frequently are also primary parasites of *Recurvaria* pupae. Males are most commonly recovered from *Copidosoma deceptor* and occasionally from *Recurvaria* pupae.

RECURVARIA PARASITES AND INSECTICIDE.—In order to forestall tree killing in the area of heavy visitor concentration around Tuolumne Meadows, 3,400 acres of the *Recurvaria* infestation were treated with an insecticidal spray during the summer of 1959. The application was contracted and supervised by the National Park Service on the basis of information provided by entomologists of the Pacific Southwest Forest and Range Experiment Station. As a consequence of this operation, biological control entomologists gained an opportunity to observe the effects of the pesticide upon natural enemies associated with *Recurvaria milleri*.

Forest Service entomologists and National Park Service personnel have found that chemical control of this pest presents unique and difficult problems; these are reported elsewhere (Struble 1955, Trostle & Eaton 1958, Sharp & Smith 1960, Stevens & Struble 1960). Only those aspects of the control program which are germane to natural enemy mortality and conservation shall be treated here.

Methods and Materials.—On the basis of pilot tests with various insecticides, Forest Service entomologists recommended the use of malathion against the needle miner. This material was applied by helicopter at 2 pounds per acre in 20 gallons of diesel fuel. The insecticide program consisted of two phases on separate areas: (1) control of newly emerged moths in July, (2) control of newly hatched larvae in early August. Comparative controls were 89.9% for the moth spray and 69.7% for the larval spray (Stevens & Struble 1960).

In order to assess the effect of malathion on *Recurvaria* parasites, the following methods were employed. At selected stations within and nearby treated plots: (1) oil sensitive cards (obtained from the Forest Service) were placed out to check spray deposit, (2) visual observations were made during, immediately following and periodically after insecticide applications, and (3) three- by six-foot catch cloths were suspended approximately 2 feet above the ground beneath treated and untreated trees. Dead insects were collected from these cloths periodically following insecticide applications.

In addition, bioassay tests were conducted with parasite species available during the period of insecticide effectiveness in the field. Parasites were obtained from uncontaminated lodgepole pine tips confined in glass-topped sleeve cages, 3 cubic feet in volume. Test insects were exposed to malathion residues on foliage collected from sprayed plots at predetermined intervals following treatment. Test units consisted of 1 cubic foot, wood frame cages with plywood tops and bottoms and cotton organdy sides. In each cage 20 lodgepole tips were suspended around all sides and placed upon the floor. Each residue test consisted of three cages containing treated foliage and one control cage containing uncontaminated foliage. Parasites were exposed to caged foliage held out-of-doors in a shaded area for approximately 36 hours. After each test the cages were decontaminated by an acetone bath. Preliminary trials proved that decontamination by this method was satisfactory and that acetone treated cages were not harmful to the parasites.

Results.—The chemically treated cards showed that spray deposits were universally adequate and generally uniform. In a few areas, however, deposit was heavier than anticipated. The cards also indicated that drift frequently occurred leeward of the sprayed areas, often for at least a quarter of a mile. Drift was also reflected in the catch cloth collections.

Visual observations were impressive because initial knockdown was devastating to practically all arboreal arthropods. A large portion of these were entomophagous species, many of which we know nothing about. For more than 2 weeks following treatment arthropod activity in the sprayed plots was noticeably reduced. Catch cloth collections were very informative, not only from the standpoint of *Recurvaria* parasite mortality but also with respect to other arthropods (associated with lodgepole pine) which will not be discussed further in this paper.

Catch cloth data for the moth control program were obtained by Forest Service entomologists who have generously allowed us to report records that we consider characteristic of the moth control operation as a whole. Dead insects from the cloths were collected periodically, dried, stored in one-half-pint paper ice cream cartons by Forest Service personnel and examined by us at a later date.

The moth spray plots reported upon here may be described as follows:

Check M. Almost pure lodgepole pine on a south facing slope.
Plot M-1. Primarily lodgepole pine with a few red firs, occasional western white pines and junipers on a south facing slope.
Plot M-2. Three-fourths lodgepole pine, one-fourth red fir on a north facing slope.
Plot M-3. Almost pure lodgepole pine interspersed with boggy meadows along the east shore of a small lake.

A similar method was applied by biological control entomologists during the spray program against the newly emerged *Recurvaria* larvae. Dead insects were collected from the catch cloths daily, stored in 70% alcohol and examined later.

The larval spray plots may be described as follows:

Check L. Predominantly lodgepole pine with some red fir at the foot of a north facing slope bordering a relatively large lake.
Plot L-1. Almost pure dense lodgepole pine on a relatively level area along a swiftly flowing river.
Plot L-2. Almost pure, rather open, lodgepole pine near the foot of a south facing slope terminating in a large meadow.
Plot L-3. Mixed lodgepole pine, western white pine, red fir and mountain hemlock on a south facing slope.

Recurvaria parasite mortality indicated by the number of individuals taken from catch cloths in moth spray and larval spray areas is presented in tables 1 and 2.

The catch cloth data disclose that all of the most effective primary parasites were adversely affected by malathion as it was applied during the control operations. This information would suggest that parasite mortality resulting from residual malathion occurred for about 10 days following the dates of application. There is little doubt that *Copidosoma* and *Dicladocerus* were reduced by the moth spray whereas *Aethecerus*, *Apanteles*, *Zagrammosoma americanum* and the more abundant hyperparasites were less affected. During the larval spray, *Copidosoma* mortality was relatively less while that of the other species was greater. *Recurvaria* has been omitted from table 2 because inconsistent recovery of the tiny first instar larvae was not truly indicative of larval mortality.

Figure 1 more clearly presents the relative mortalities of *Recurvaria* and *Copidosoma* indicated by catch cloth collections during the moth control program. Moth kill was initially great but decreased sharply until, after about 1 week, numbers of moths recovered were of little consequence. In figure 1 on the graph for the check, moth mortality between July 17 and 23 could have been caused by slight drift from treated areas nearby. Similarly, in unit M-2 mortality prior to the date of application also could have been caused by slight drift. On the graphs for units M-2 and M-3, the increases in moths recovered on July 31 are misleading. Moths recovered on that date represent a 3-day accumulated mortality (a maximum of 46 in plot M-2). Had these collections been made daily, an increase in moth recovery, if any, would have been much less obvious. In unit M-2 particularly, a gradual decline in daily recovery of moths would probably represent a more accurate picture. Thus, a significant increase of moth emergence and mortality between July 28 and 31 is

Table 1.—*Recurvaria* and parasite mortality during the moth spray program as indicated by numbers of individuals recovered from catch cloths.[a]

Species	Plot No.	Numbers Recovered														
		July												August		
		15	16	17	18	19	20	22	23	24	27	28	30	31	3	5	7
Recurvaria milleri	M-Check	‡	‡	12	22	3	8	‡	17		3		‡				
	M-1	‡	581*	82	39	30	12	‡	‡	12	11	‡	6	‡	3	3	‡
	M-2	2	28	18	37	25	44	123*	354	47	34	30	‡	46	8	8	3
	M-3	‡	1	2	4		3	9	227*	410	2	6	‡	19	7	3	2
Copidosoma deceptor	M-Check	‡	‡					‡	1		3		‡	1	6	2	34
	M-1	‡	42*	7	5	5	8	‡	‡	10	7	‡	153	‡	49	11	‡
	M-2				1			17*	13	7	34	3	‡	280	101	26	63
	M-3	‡						2	3*	3	15	4	‡	103	46	33	86
Apanteles californicus	M-Check	‡	‡					‡					‡				
	M-1		*					‡	‡			‡		‡			
	M-2							*		1			‡				
	M-3	‡										1	‡				
Dicladocerus sp., 84.71%	M-Check	‡	‡		1			‡					‡				
Sympiesis stigmatipennis, 1.18%	M-1	‡	30*	2	2	2		‡	‡			‡	‡				‡
Chrysocharis, 11.76%	M-2							5*	1	2			‡				
Other, 2.35%	M-3	‡							8*	4	1		‡	3			
Zagrammosoma americanum	M-Check	‡	‡					‡					‡				
	M-1		*					‡	‡			‡		‡			‡
	M-2							*					‡				
	M-3	‡						1	*				‡				
Aethecerus pinifolii	M-Check	‡	‡					‡					‡				
	M-1	‡	*					‡	‡		‡	‡		‡			‡
	M-2							*					‡				
	M-3	‡							4*				‡				
Amblymerus sp.	M-Check	‡	‡					‡					‡				
	M-1	‡	3*	1	2		1	‡	‡			‡	‡	‡	3	2	‡
	M-2						1	*					‡				
	M-3	‡							1*	1		1	‡	1			
Zagrammosoma nigrolineatum	M-Check	‡	‡					‡					‡				
	M-1		3*					‡	‡			‡		‡			‡
	M-2					1		2*					‡		1		
	M-3	‡							*				‡				

[a] *, date sprayed; ‡, not collected on this date.

Table 2.—Parasite mortality during the larval spray program as indicated by numbers of individuals recovered from catch cloths.[a]

Parasite Species	Plot No.	Numbers Recovered																			
		August													September						
		18	19	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	8	10
Copidosoma deceptor	L-Check	1	1	5	1	1		1	1		1	1		1	2			1	1			1	1
	L-1	‡	17*	21	6	3				1	1			‡		1						2	
	L-2	‡	‡	2*	10	1	1	1						1	1		2						
	L-3	31†	28	9	5†	42*	9	2								1	1						1
Apanteles californicus	L-Check				1					1		1									1		
	L-1	‡	6*	2						1				‡						1			
	L-2	‡	‡	2*	8						1												
	L-3	2†	2		†	1*									1	2							
Dicladocerus sp.	L-Check			2			1								1								
	L-1	‡	33*	17	3	1				3	1	1	1	‡			1						
	L-2	‡	‡	1*	2		2	3		1	2	1		1				1	1				
	L-3	10†	10	6	16†	73*	18	6	3	2	2	3	3	4	7	3	4	3	2	1	2	4	1
Sympiesis stigmatipennis	L-Check																						
	L-1	‡	7*	3										‡									
	L-2	‡	‡	1*	9	3	1		1	1	2												
	L-3	1†	1		†	*	5											1	1				
Zagrammosoma americanum	L-Check																						
	L-1	‡	*	2	1						1												
	L-2	‡	‡	*	3		1			1													
	L-3				†	4*	10	1	1								2			1			
Aethecerus pinifolii	L-Check			1												1							
	L-1	‡	65*	13	5	39	2	7	1	9	7	4	1	‡	1	1	1	2					
	L-2	‡	‡	*	13	1	1	1												1			1
	L-3	1†	1		†	3*	4																
Amblymerus sp.	L-Check	2	1					1			1	1	1	2	1		1				2	3	
	L-1	‡	38*	16	3	2		2		2	1	2	2							1	1	2	
	L-2	‡	‡	*	11	3		1				1	2										
	L-3	51†	49	20	12†	39*	22	3	1				1		1		3	1		3		5	1
Chrysocharis sp.	L-Check							2	2	1				1								1	2
	L-1	‡	10*			4	1		2					‡	1	1	2		1	4	2	1	
	L-2	‡	‡	1*	17			3	2	1	1	2	1	3		1		1		1			1
	L-3	5†	5	2	†	15*	12	1	3	3	6	4	1	10	2	7	6		3	3	5	17	8
Tetrastichus sp.	L-Check			3	1														1				
	L-1	‡	*	2	1					2	1			‡		1	1						
	L-2	‡	‡	1*	29	2							1										
	L-3	8†	8		2†	15*	4	1			1				1	1	1				1		
Zagrammosoma nigrolineatum	L-Check																						
	L-1	‡	6*	4	1	1				1		1		‡				1				1	
	L-2	‡	‡	*	2			1															
	L-3	1†	1	1	†	6*	13	1	1						1			2					

[a] *, date sprayed; †, drift; ‡, not collected on this date.

unlikely to have occurred. These analyses suggest that maximum moth emergence in the general treatment area had occurred by July 20 and had essentially terminated prior to the times of application in most spray units. Additional data concerning *Recurvaria* emergence during the moth spray program are presented by Stevens & Struble (1960). Invasion of treated plots by moths from untreated areas was so slight that it could not be detected in catch cloth recoveries nor by visual observation.

Because the results of bioassay tests will show that *Copidosoma* is readily killed by malathion-oil spray (fig. 2), it may be concluded that the parasite mortality peaks in figure 1 also represent peak periods of *Copidosoma* emergence. In all three sprayed units *Copidosoma* emergence did not peak until the end of July, at least 10 days after the moth emergence had attenuated. Note how *Copidosoma* mortality peaks hold a similar position in all graphs while *Recurvaria* mortality peaks move closer to them as the application dates become later. During the larval spray program, after *Copidosoma* had essentially completed emergence, its mortality peaked immediately following spray application (table 2). If *Copidosoma* emergence had climaxed before the dates of the moth control application (as is the case with the needle miner) peak mortalities for the pest and the parasite would have been superimposed in figure 1. The fact that *Copidosoma* emergence normally occurs at least 10 days after that of *Recurvaria* is substantiated by these observations.

A few mature needle miner larvae occurred in catch cloths. These larvae did not signify that moth emergence would have continued. Other investigations have shown such larvae to be parasitized by *Apanteles californicus*. Twenty-five tardy larvae were dissected; all were parasitized. Apparently most of these parasitized larvae remained within needle mines during and after the moth spray. Should this be the case, it is probable that many of the *Apanteles* completed development unharmed, even though some of the host larvae were killed. A similar situation has been reported for *Apanteles fumiferanae* Vier. as a result of DDT applications against the spruce budworm in Canada (MacDonald 1959).

The parasite bioassay tests suffered from some necessary limitations. Most notable of these were insufficient manpower, time and equipment. As a consequence, low numbers of certain parasite species were used in these tests. The variability of the tests with respect to the numbers of each parasite species was dictated by the abundance of the test insects available in the rearing

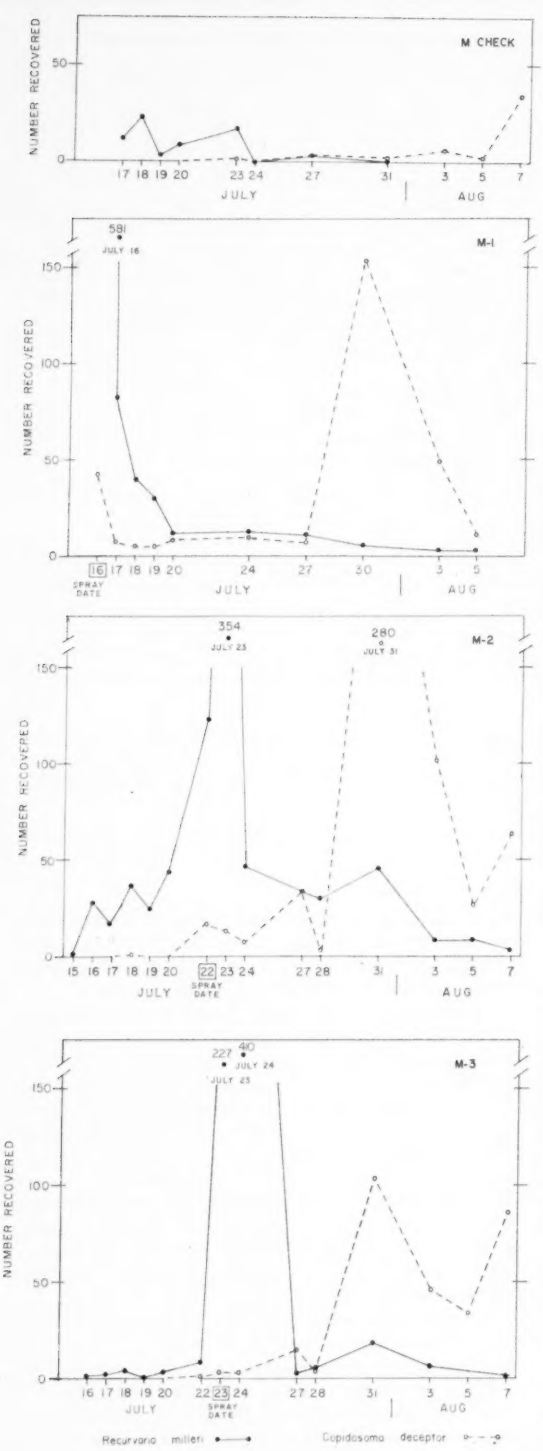

Table 3.—Numbers of individuals of parasite species subjected to malathion residue bioassay tests during programs for control of moths and first instar larvae of *Recurvaria milleri*.

Species	Total in		No. of Individuals in Residue Cages	
	Combined Controls	All Residue Cages	In All Tests (range)	Per Test (median)
Moth spray				
Copidosoma deceptor	214	589	43–60	55
Aethecerus pinifolii	66	200	9–28	14
Dicladocerus sp.	71	201	10–26	11
Larval spray				
Aethecerus pinifolii	30	72	5–17	9
Dicladocerus sp.	19	55	4–12	8
Zagrammosoma americanum	21	48	2–12	4
Amblymerus sp.	74	174	6–38	19

cages on the days that the tests were conducted.

Controls for all the tests of each parasite species were combined and were found to constitute homogeneous populations (by X^2 at the 5% level of significance). Therefore, in the analysis of each test, the mortality in the three residue (treated) cages was compared with the mortality in the combined controls of all the tests of the series. Thus, mortality caused by malathion residue on selected dates following application was compared with the combined controls also by the X^2 method at the 5% level of significance. This procedure was followed in the moth control and the larval control programs. Each of these programs is represented by a distinct series of tests and analyses.

For each parasite species bioassayed, the following information is shown in table 3: total number of individuals in the combined controls, total number of individuals in all residue (treated) cages, range of individuals in residue (treated) cages in all tests and the median number of individuals in the residue (treated) cages per test.

Parasite mortality caused by malathion-contaminated foliage on selected days following treatment is shown in figure 2.

The data in figure 2 indicate that significant mortality of *Copidosoma* occurred consistently for 16 days following application. Unfortunately, tests were terminated at that time due to a shortage of this parasite. *Aethecerus*, *Dicladocerus*, *Zagrammosoma americanum* and *Amblymerus* were killed consistently from 8 to 13 days following malathion application. Irregular mortalities recorded for the later tests are attributed to some variation in spray deposits from tree to tree and area to area. Inasmuch as contaminated tips were collected from the mid-crowns of more than one tree in a treated area and from more than one plot, some variation in older residues might be expected.

A noteworthy feature of the mortality histograms is their bimodal distribution. There is a decidedly reduced parasite kill at 4 to 5 days following application. In fact, *Aethecerus*, *Dicladocerus* and *Zagrammosoma* were unaffected at this time during the larval control program.

FIG. 1.—Relative mortalities of *Recurvaria* moths (solid line) and *Copidosoma* adults (dotted line) as indicated by individuals recovered from catch cloths in three moth-spray areas.

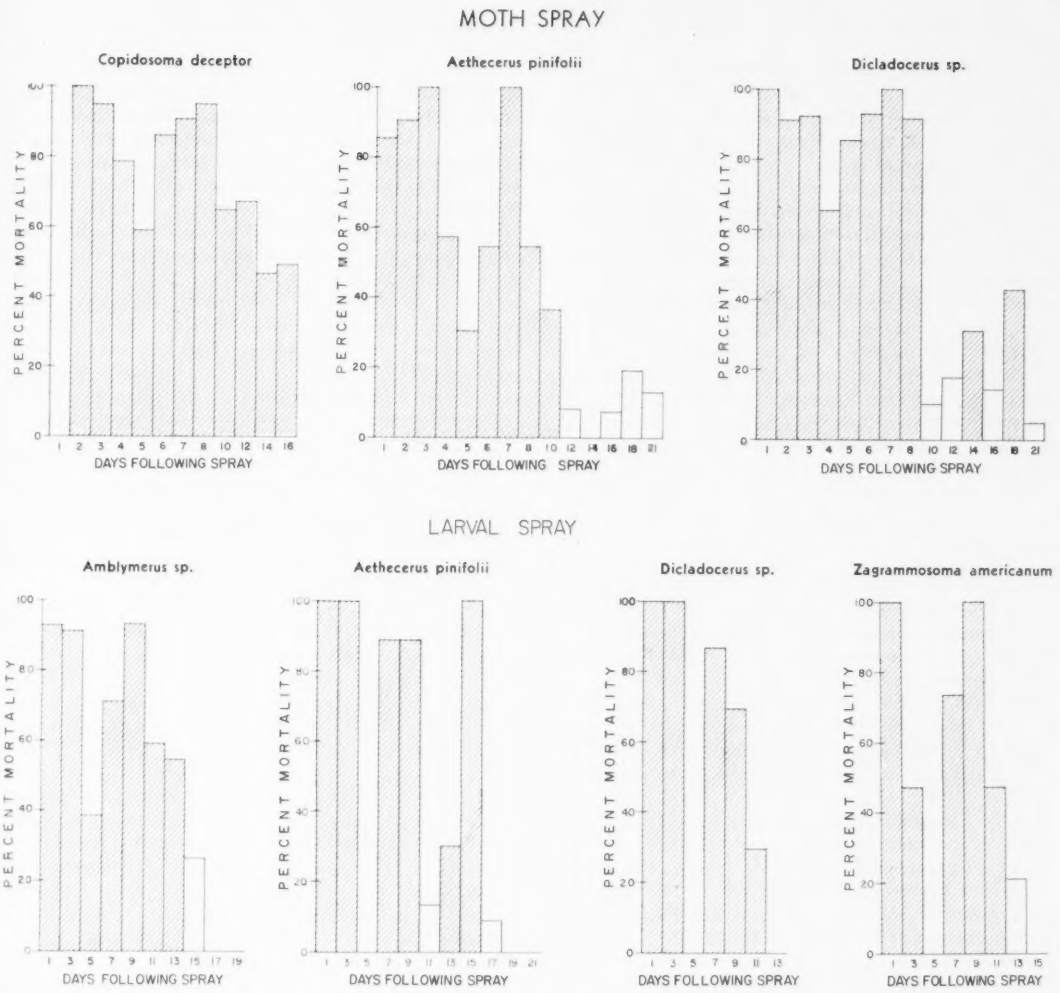

FIG. 2.—Parasite mortality caused by malathion-contaminated foliage in cage tests on selected days following treatment.
▨ = significant at .05 level with X^2 analysis.
□ = not significant.

Such ostensively aberrant results during the moth spray prompted repetition of the 5-day tests. In spite of this second trial, results were essentially the same. Thus, a reduced parasite mortality was evident for all species at 4 to 5 days following application on three occasions, twice during moth spray tests and once during the larval spray tests. As there was no clue to the cause of this unexpected pattern of parasite mortality, we have no explanation for it. It is possible that chemical changes occur in the malathion-oil deposits at this time. However, chemical analysis of residues which might have disclosed such changes were not available during the spray programs.

Attempts to subject *Apanteles californicus* to bioassay were unsuccessful. The parasite was relatively scarce in the rearing cages and did not tolerate the conditions of the experiment, even in the control cages. Other parasite species were not tested because they were in short supply during the periods of the tests.

For the parasite species tested, bioassays compare favorably with the catch cloth records. The cage tests, however, probably provide for a more accurate estimate of effectual malathion residue because it is more certain that parasites were present and contacted contaminated tips throughout the experimental period.

Discussion.—Earlier reports record up to more than 40% parasite-induced mortality of *Recurvaria* during the course of the current outbreak (Struble 1958b, Struble & Martignoni 1959). Whether or not this percentage will increase remains to be seen. Nevertheless, it is advantageous to conserve and encourage mortality factors which can reduce a pest population by more than two-fifths. Although such mortality may not terminate an outbreak,

the rate of pest increase is certainly reduced by it. Data presented here have substantiated the observation that malathion-oil applications killed the principal parasites of the lodgepole needle miner. It was unfortunate in this case, as it has been in others, that natural enemy investigations were unavoidably initiated in the late stages of an outbreak. Consequently, it was not possible to give greater consideration to parasite conservation during the planning stages of the recent control program.

Information gained during needle miner control operations coupled with a still limited understanding of pest-parasite ecology now may allow for greater natural enemy conservation if insecticide applications against *Recurvaria* are again necessary. External larval parasites are of extremely low incidence on *Recurvaria* during the off-flight years. Pupal parasites are then in alternate hosts and the synchronized species are immature within needle miner caterpillars. From the standpoint of parasite conservation, an insecticidal control program against this pest should be most advantageous during these off-flight years. There would be a chance that synchronized parasites would be killed within their hosts but per cent parasitization by these species in the surviving host population probably would not be greatly changed. A safe insecticide which is effective against the needle miner in the off-flight years has not been found. However, the search for such a material has been intensified. Presently, the remaining alternatives are control programs against the moths and newly hatched larvae.

Catch cloth records indicate that pupae of the principal primary parasites are unharmed (parasite emergence occurred subsequent to insecticide application). These data and earlier investigations have revealed that the greatest numbers of the principal parasite species emerge 10 days or more later than the moths. It seems, therefore, that a moth control program would kill fewer parasites than a program against the newly hatched larvae. Both kinds of programs killed *Recurvaria* during the summer of 1959. However, the relative capacities of the surviving pest populations for renewal of the outbreak in treated areas remain to be seen.

Should an identical moth control program be adopted, *Aethecerus* (the chief pupal parasite), *Apanteles* and many of the eulophids might escape serious injury. On the other hand, *Copidosoma* and early emerging eulophids would still sustain sizable losses. Yet, with certain innovations a moth control program might be planned so that *Copidosoma* and eulophid losses could be lessened.

On the basis of the foregoing information it is reasonable to assume that, had the insecticide been applied a week or more earlier, an equally effective needle miner reduction would have been accomplished—but more *Copidosoma* and early emerging eulophids would have been spared. Perhaps an insecticide applied at about the 50% level of moth emergence and formulated to have an effective residue no longer than 8 or 10 days might have been particularly beneficial in this regard.

Would a precisely timed insecticide with a limited residual period allow escaping moths to renew an outbreak? Information gained from fundamental needle miner-parasite investigations indicates that this approach might be effective and perhaps more lasting. Catch cloth records show that a shorter residual period would probably allow a relatively small number of late emerging moths to escape. However, more than 80% of these would be females (Struble 1958b). According to Morgan 1956, both males and females usually mate only once. At a ratio of one male to four females, only one-fourth of the escaping females would be fertilized, even if every male found a female and successful mating was accomplished. This is a generous estimate because Morgan also noted that mating appeared to depend upon the concentration of males and females within a small area such as foliage tip. In the field, mating did not begin until the per tip population had risen to about four to six individuals. Thus, it is probable that less than 20% of the surviving moth population would have the potential for perpetuating the outbreak. If every fertilized female could lay a maximum complement of eggs (estimates are 40, Morgan 1956, to 56, Struble 1954), these eggs would be subjected to an overwhelming population of *Copidosoma* spared from insecticide residue. After hatching, each young larva would be subjected to a much more favorable ratio of *Apanteles*, and so on. In addition, abiotic mortality factors would also effect a reduction of these remaining progeny. The risk of a rapid buildup of *Recurvaria* under these conditions does not seem great.

Should preliminary tests demonstrate this approach to be feasible, its application has still another advantage. Neither the pest nor its parasites would be eliminated from treated areas. Rather, a more favorable pest-parasite ratio would be established. Thus, a situation favorable to more sustained biological control of the needle miner should be created.

As more insecticides are tested against the lodgepole needle miner, perhaps one of less general arthropod toxicity than malathion will prove to be acceptable. Should a material which is more selective against Lepidoptera than Hymenoptera meet the requirements for needle miner control, parasite survival might be considerably enhanced. Inasmuch as such insecticides are available, there is some hope that they may be useful against this pest.

Delineation of treatment units is another aspect of forest pest control which impinges upon the maintenance of entomophagous species. It is generally believed that the closer the centers of treated areas are to untreated areas, the better will be the chance of natural enemy reestablishment. Unit size and layout of the recent needle miner control program was ideal in this regard. Most units were relatively small and peninsular-like in shape. Thus, regardless of parasite kill in treated areas, a renewal stock might be available to them. On the basis of this principle, a method of alternating treated with untreated strip blocks is receiving more attention in food crop entomology. Perhaps an analogous method will find application in certain kinds of forest pest control problems.

By testing and possibly adopting some of the suggestions presented here, we feel that greater *Recurraria*-parasite conservation might be achieved. In fact, it is quite possible that host-parasite ratios in treated areas might be shifted so that the beneficial species would outnumber the pest. In this way, chemical control would be

effectively augmented and a more equitable, perhaps relatively more permanent, parasite-host balance might be reestablished.

Conclusions.—A malathion-oil spray program against the lodgepole needle miner in Yosemite National Park was detrimental to the defoliator's parasites. Through information gained during the control program, augmented by a knowledge of fundamental pest-parasite ecology, it may be possible to achieve greater natural enemy conservation in future control programs.

None of the needle miner parasites displayed sufficient resistance to the insecticide so as to escape kill in treated areas. It is probable that very few silvic entomophagous species are physiologically resistant to insecticides currently applied against forest pests. Beneficial forms reported as unharmed by control programs, more likely, have escaped contact with the insecticide. It is expedient, therefore, to understand how and why forest parasites and predators are affected by control programs. When this aim is achieved, it may then be possible to encourage the conservation of important entomophagous species. A major prerequisite to gaining this potential advantage is a more thorough knowledge of what forest pests' natural enemies are and what they do.

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Field Experiments on Insecticidal Control of Insect Pests of Cabbage and Broccoli[1]

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ABSTRACT

Weekly applications of Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) or Dibrom® (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate) gave better control of cabbage looper, *Trichoplusia ni* (Hbn.), and imported cabbageworm, *Pieris rapae* (L.), based on percentage of undamaged cabbage heads harvested than Methyl Trithion® (*O,O*-dimethyl *S*-(*p*-chlorophenylthio) methyl phosphorodithioate), phosphamidon, Kepone® (decachloroöctahydro-1,3,4-metheno-2*H*-cyclobuta[cd] pentalen-2-one) or methoxychlor. Against cabbage looper on broccoli, dimethoate, Dibrom, or Thiodan followed by combinations of Dibrom and Perthane® (1,1-dichloro-2,2-bis(*p*-ethylphenyl)ethane), and malathion and Perthane, gave higher reduction of cabbage looper in the heads at harvest than Methyl Trithion, phosphamidon, Thiodan (at one-half recommended dosage) or Am. Cyanamid 24055 (1,1-dimethyl-3-(*p*-acetamidophenyl) triazene). The latter, an antifeeding compound, significantly reduced feeding injury over untreated plots. Dimethoate is a particularly promising insecticide for control of cole crop insects.

As a pest of processing crops in Maryland the cabbage looper, *Trichoplusia ni* (Hbn.), is second only to the corn earworm, *Heliothis zea* (Boddie). In recent years heavy infestations of this looper have been observed on tomatoes, peppers, spinach, mustard greens, kale, cabbage, broccoli, and cauliflower. Not only is great damage done to these crops, but the larvae are difficult to remove during processing and are a contamination hazard in the canned or frozen product. Larvae of the cabbage looper have been successfully removed from broccoli by a dipping or a quick wash in warm (80° to 100° F.) pyrethrum solution (1 pint 2% concentrate in 100 gallons of water), followed by tumbling in a sand reel (a large, rotating, wire cylinder) and a prolonged wash in clean water. The pupae can be removed only by hand since they are attached tightly to the heads or leaves by strong webs. The pupae of the diamondback moth, *Plutella maculipennis* (Curt.), present a similar problem on leafy crucifers. Some processors refuse to handle kale or mustard greens with as few as two or three cabbage looper or diamondback pupae per ton of raw product. On leafy crops pupae are difficult to see and cannot all be eliminated from the inspection belt with assurance.

The problem of controlling the cabbage looper is difficult because of the resistance of larger larvae to nearly all of our available insecticides and the necessity of maintaining chemical residues within legal tolerances during periods of harvest. To kill small larvae, which is necessary for commercially controlling this insect, repeated applications of short-lived insecticides must be made. Weather often causes interruption of spray schedules. Considering these factors, the best control would appear to be the use of a protective schedule consisting of the most effective short-lived insecticide combined with the most effective persistent material with a high legal residue tolerance.

Even with the most effective insecticides under favorable weather conditions, heavy looper infestations are extremely difficult to eliminate. Satisfactory commercial control has been possible in Maryland only because the looper populations have been held at a low level during the late summer and fall by the polyhedrosis virus of the larvae. This virus has naturally occurred throughout our broccoli-growing area for the past 3 years.

Two other factors contributing to the problem are the difficulty of obtaining adequate insecticidal coverage of the leafy crops involved, and the development of resistance by the larvae of the imported cabbageworm and the cabbage looper to much-used insecticides, as evidenced by reports from Wisconsin (McEwen & Chapman 1952) and New York (Hervey & Swenson 1953).

A continuous search for more effective insecticides and combinations of insecticides must be made if we are to continue growing broccoli for processing. Since the polyhedral virus of the looper larvae has not occurred at College Park, Md., it was desirable that investigations be carried on here. This was done on both cabbage and broccoli in 1959. Though cabbage looper control has been the primary objective of these experiments, observations have also been made on other pests of cabbage and broccoli.

CABBAGE EXPERIMENT.—The Golden Acre variety of cabbage was set in the field June 15 in 4-row plots 80 feet long. Six insecticides were applied weekly in water at the rate of 50 gallons of finished spray per acre with an improved or "Slosser" type spray boom (Burkhardt & Ditman 1956) equipped with six nozzles per row. There were two hollow-cone nozzles on each side of the row directed inward and two flat-spray nozzles over the plants directed downward. All treatments were applied in randomized blocks replicated three times. Six applications of all insecticides except phosphamidon were made, the first on June 22 and the last on August 3. The last application of phosphamidon was omitted to obtain residue samples at a desired period after the last application. Plants were examined for feeding injury on July 17 and 30. On July 17 every third plant in each plot was examined for the presence of insects. Heads were harvested on August 3 and 10. Number, weight and condition of heads and insects present were recorded. Nature of treatments and results are given in table 1.

The cabbage was attacked during its growing season by a cabbage flea beetle, *Phyllotreta cruciferae* (Goeze), the imported cabbageworm, the cabbage looper, and the cabbage aphid, *Brevicoryne brassicae* (L.). During the early growing season the imported cabbageworm popula-

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Table 1.—Results of insecticides applied to cabbage in 1959. College Park, Md.

Treatment (pints/A.)	Toxicant/A. (lb.)	July 17: Plants Showing Feeding Injury (%)	July 17: Insects per 10 Plants: Imp. Worm	July 17: Insects per 10 Plants: Looper	July 17: Insects per 10 Plants: Flea Beetle	Number of Heads: No. Heads Harvested	Number of Heads: Clean (%)	Number of Heads: Clean Weight (lb.)	Per Cent of Heads: Infested with Aphid	Per Cent of Heads: Injured by Worms	Worms Recovered: Imp. Worm	Worms Recovered: Looper	Flavor Value[a]
Dibrom, 1	1.00	24.4	1.0	0	0.9	148	70	100.5	15.5	16.8	2	0	3.7
Thiodan, 3	0.75	7.6	0.7	0	.2	212	93	244.2	0	7.1	0	0	4.2
Methyl Trithion, 1.5	.75	13.2	0.5	2	.2	182	37	73.7	11.5	57.1	5	0	3.9
Phosphamidon, 1	.50	9.0	0.4	4	.2	196	55	141.0	3.0	41.3	0	3	3.8
Kepone, 4	1.00	46.8	1.7	0	.3	165	42	61.3	39.3	33.9	0	8	3.5
Methoxychlor, 4	1.00	22.1	0.4	0	.2	202	48	110.0	19.3	35.2	1	1	3.9
Check, —	—	38.3	4.6	0	4.1	179	9	13.2	69.2	73.7	1	9	4.0

[a] Differences not significant at 5% level.

tion was much higher than the looper population but by harvest time the looper became more prevalent. The cabbage aphid was abundant during the entire season. In these tests at the dosages used, Thiodan® (6,7,8,9,10,10,-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) treated plots outyielded those of other treatments and gave best control of all insects present. Based on per cent of clean heads, Dibrom® (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate) rated second in order of performance.

TEST ON BROCCOLI.—The Walton 29 variety of broccoli was set in the field August 11 and 12 in 4-row plots 40 feet long. A single row was left unplanted between plots so that they were approximately 6 feet apart. Twelve treatments including an untreated control were randomized in blocks which were replicated four times. The entire plots were treated in all instances but all observations were made and results taken only on the two inside rows of each plot. Eleven weekly applications of all treatments, except treatment 11, were made beginning August 25 and ending November 4. Am. Cyanamid 24055 (1,1-dimethyl-3-(*p*-acetamidophenyl)triazene)was not available for the August 25 application so that the first application of this material was made September 1. Treatments 8 and 9 were changed to the late spray schedule (as indicated in table 1) on September 21.

On August 25, September 1 and 7, all insecticides were applied in water with an improved or "Slosser" type boom equipped with five nozzles per row (Burkhardt & Ditman 1956), at the rate of 50 gallons of finished spray per acre. On September 14, because plants were larger, the amount of water was increased to 100 gallons per acre. The amount of insecticide per acre was maintained at a constant rate for all insecticides used. To obtain the 100 gallon per acre rate of spraying, a pea broadcast boom (Ditman & Burkhardt 1952) was added behind the improved boom, a practice called "double booming." "Double booming" appears to be desirable on broccoli during the latter half of the growing season when it is necessary to protect the heavy rapidly developing foliage in the top portion of the plant.

Records on populations of the imported cabbageworm, cabbage looper and diamondback moth were taken September 18 on a 10-plant sample in each plot. Larvae were recorded as small (less than half grown) and large. Nature of treatments and the insect populations recorded are given in table 2. Since only six imported cabbageworm larvae and one diamondback moth larva were found on untreated plants, the records on these species are not included in table 2. Plots treated with dimethoate at 0.75 and 1.0 pound and Dibrom at 1.0 pound per acre showed the lowest number of loopers present.

The center heads of broccoli were harvested, counted, weighed and examined for the presence of insects. Four harvests were made at approximately weekly intervals, the first October 8 and the last November 6. Samples from the last harvest were frozen and evaluated for flavor. Results are given in table 3. Only three heads from the check plots were found to be infested with aphids and therefore the data taken on the cabbage aphid were omitted from the table. The lowest number of cabbage looper larvae was found on heads treated with dimethoate at 1.0 pound, and those to which the thiodan-Dibrom-Perthane schedule was applied. Ninety per cent or more clean heads were obtained with dimethoate (at 0.75 and 1.0 pound per acre), Dibrom, and treatments of Thiodan followed by Perthane®(1,1-dichloro-2,2-bis(*p*-ethylphenyl)ethane) and Dibrom, and Perthane and malathion.

On October 27 plots were rated for appearance and amount of general worm-feeding injury. The plants in the two inside rows of each plot were counted and records were made of the number of clean, slightly injured (small holes in leaves indicating larvae had not reached maturity), moderately and severely injured plants. Numerical values of zero to 3 were assigned these classes of injury.

Table 2.—Cabbage looper infestation on broccoli, September 18, 1959.

Treatment (pints/A.)	Actual Toxicant/A. (lb.)	Number of Cabbage Loopers on 40 Plants[a]: Small	Large	Pupae	Total
Dimethoate, 1	0.50	13	10		23
1.5	0.75	1		1	2
2	1.00	6	1	2	9
Methyl Trithion, 2	1.00	11	6	1	18
Phosphamidon, 1	0.50	33	5	11	49
2	1.00	11	4	7	22
Dibrom, 1	1.00	7		2	9
Thiodan, 0.75 followed by Malathion, 2.0 and Perthane,[b] 2.0	0.38 1.25 1.00	38	8	4	50
Thiodan, 0.75 followed by Dibrom, 1.0 and Perthane,[b] 2.0	0.38 1.00 1.00	29	3	7	39
Thiodan, 0.75	0.38	27	7	3	37
Am. Cyanamid 24055, 8.0 (lb.)	2.00	10	1	5	16
Check, —	—	62	19	8	97

[a] Loopers counted on 10-plant samples in each of four replications.

[b] Thiodan applied till edible parts formed, 4 applications, followed by 8 applications of malathion-Perthane or Dibrom-Perthane sprays up to and during harvest period. Same treatment also in tables 3 and 4.

Table 3.—Results of insecticide treatments against imported cabbageworm, cabbage looper and cabbage aphid in harvested broccoli, and flavor evaluation of processed and frozen product.

Treatment and Actual Toxicant/A. (lb.)	Total Heads			Per Cent Clean Heads[a]	Total Number of				Flavor Value[b]
	No.	Weight (lb.)	Clean		Imported Cabbageworm	Heads Infested by Cabbage Looper	Heads Infested by Aphids	Worms in Heads Harvested	
Dimethoate, 0.50	228	50.9	203	71.5	1	25	0	29	3.9
Dimethoate, 0.75	177	42.0	168	77.5	2	8	0	11	4.1
Dimethoate, 1	192	47.3	185	81.1	1	6	0	8	3.9
Methyl Trithion, 1	222	49.9	150	39.9	1	72	0	94	3.9
Phosphamidon, 0.5	203	47.4	134	54.9	0	69	0	102	3.9
Phosphamidon, 1	181	40.7	113	51.1	1	68	0	100	4.0
Dibrom, 1	216	52.5	195	71.9	0	21	0	24	3.8
Thiodan, 0.37 followed by malathion, 1.25 and Perthane, 1	230	49.9	212	73.8	0	18	0	19	3.9
Thiodan, 0.37 followed by Dibrom, 1 and Perthane, 1	194	45.8	186	79.7	0	8	0	8	4.0
Thiodan, 0.37	201	46.1	167	66.4	0	32	0	39	3.9
Am. Cyanamid 24055, 2	166	34.5	134	64.6	1	32	0	37	3.9
Check	210	43.8	126	49.3	11	84	3	120	3.8

[a] Transformed to angles—L.S.D.@ 5% = 9.1.
[b] Differences not significant at 5% level.

An injury index was then calculated by the following formula:

$$\frac{(\text{No. plants severe} \times 3) + (\text{No. plants moderate} \times 2) + (\text{No. plants slight} \times 1)}{\text{Total No. of Plants}} = \text{injury index}$$

These results are given in table 4. They confirm the relative effectiveness of treatments as determined by percentage of clean heads and number of larvae recorded from the harvested heads more closely than larval counts on younger plants. Larvae are difficult to find on plants and such counts are probably the least accurate method of estimating insecticidal efficiency.

Additional estimates of the populations of the imported cabbageworm and cabbage looper were made on the plots receiving the anti-feeding compound, Am. Cyanamid 24055. Examinations of 10 plants on each treated plot and on an untreated check were made September 1, 3 and 9. The first application of Am. Cyanamid 24055 was made September 1 and a second was made September 7. Results are given in table 5. The rapid reduction of the cabbage looper following the first treatment indicates some toxicity of the compound to the cabbage looper though it is possible that the larvae merely moved from the treated plants.

In addition to the main experiment, single applications of several insecticides and combinations of insecticides were made to plots in a block which had not been treated previously. These insecticides were applied, in duplicated randomized blocks, at heavier dosages or in combinations not applied in the other experiments to determine if a population of the cabbage looper could be eliminated with one spray. Cabbage looper larvae were counted on two 10-plant samples in each plot on September 14 just before treatments were applied, and again on September 17 and 81 (3 and 4 days after spraying). The larvae were counted

Table 4.—Injury rating of imported cabbageworm and cabbage looper to foliage of broccoli, October 27, 1959.

Treatment	Total Plants (no.)	Injury Rating (see text)				
		Severe (%)	Medium (%)	Slight (%)	None (%)	Index
Dimethoate	268	0.0	21.6	64.2	14.2	1.07
	269	0.0	1.1	72.9	26.0	.75
	265	0.0	0.0	57.7	42.3	.58
Methyl Trithion	268	0.8	27.6	63.8	7.8	1.21
Phosphamidon	268	13.4	48.1	35.5	3.0	1.72
	267	14.6	48.7	31.5	5.2	1.73
Dibrom	269	0.0	5.2	63.6	31.2	.74
Thiodan followed by malathion & Perthane	261	0.0	1.9	59.0	39.1	.63
Thiodan followed by Dibrom & Perthane	252	0.0	0.4	55.6	44.0	.56
Thiodan	276	2.2	17.3	68.5	12.0	1.10
Am. Cyanamid 24055	264	1.1	22.4	70.1	6.4	1.18
Check	277	90.3	9.7	0.0	0.0	2.90

Table 5.—Imported cabbageworm and cabbage looper found on broccoli plants treated with Am. Cyanamid 24055.

Plot	Date of Examinations					
	Sept. 1[a]		Sept. 3		Sept. 9	
	Imported Cabbageworm	Cabbage Looper	Imported Cabbageworm	Cabbage Looper	Imported Cabbageworm	Cabbage Looper
Treated	0	19	0	7	0	7
Check	1	29	1	16	0	17
Treated	0	16	0	3	0	2
Check	2	12	1	14	0	15
Treated	1	29	0	12	0	4
Check	1	25	0	23	0	24
Treated	0	3	0	3	0	0
Check	6	20	1	25	1	21
Tot. Am. Cyn.	1	66	0	25	0	13
Tot. Check	10	86	3	78	1	77

[a] Counts made on 10-plant samples in each plot immediately before first application of Am. Cyanamid 24055, on September 1, again on September 3, 48 hours after treatment and on September 9, 48 hours after second application. Both larvae and pupae included in totals.

Table 6.—Effect of single applications of some insecticides and combinations of insecticides against cabbage looper on broccoli.[a]

Treatment (Pints/A.)	Actual Toxicant/A (Lb.)	Number Cabbage Looper Larvae on 40 Plants on					
		Sept. 14		Sept. 17		Sept. 18	
		Small	Large	Small	Large	Small	Large
Dimethoate, 4.0	2.0	32	11	2	2	1	0
Endrin, 1.25 plus parathion, .5	.25 .25	51	3	0	0	0	0
Kepone, 4.0	1.0	18	2	3	1	4	2
Thiodan, 4.0	1.0	40	3	2	0	0	0
Thiodan, 4.0 plus parathion, .5	1.0 .25	41	4	8	8	6	7
Check	—	31	4	17	3	12	2

[a] Treatment applied September 15.

on the same plants each time and recorded as small or large. The nature of the treatments and the results of the counts are given in table 6. Endrin plus parathion killed all larvae present within 48 hours. Thiodan alone at 1 pound of active ingredient per acre reduced the population of the cabbage looper to zero within 72 hours whereas, for reasons not understood, the addition of 0.25 pound of actual parathion to Thiodan resulted in a reduced kill of cabbage looper larvae. A similar condition was previously observed when malathion was combined with Kepone® (decachloroöctahydro-1,3,4-metheno-2*H*-cyclobutal[cd]pentalen-2-one), the combination being less effective against the cabbage aphid than when malathion was applied alone (Steinhauer *et al.* 1959).

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Techniques for Determining the Toxicity of Pine Resin Vapors to *Dendroctonus brevicomis* and *D. jeffreyi*[1]

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Abstract

Several procedures were explored to obtain a suitable technique for determining the fumigant toxicity of pine resin vapors to adult bark beetles. The most suitable fumigation chamber used was a 150-cc. screw-cap jar with a teflon plastic gasket, though a cork-stoppered 30-cc. test tube had certain limited uses. Beetles were held within the chamber in individual cells of glass and lumite screening. Fresh resin, collected from trees by a microtap device, was apportioned by volume, and the dosage of vapor in a chamber was determined by changes in weight of the resin sample.

A saturated atmosphere of resin vapor was reached within 24 hours after placing a resin sample in the chamber. A 150-cc. volume was saturated by approximately 2 mg. of vapor of *Pinus ponderosa* Laws. resin, by 20 mg. of *P. jeffreyi* Grev. & Balf., and by 6 mg. of *P. jeffreyi*×*ponderosa*.

Using *Dendroctonus brevicomis* Lec. as the test insect, it was found that light, evaporative area of resin, and volume of the chamber did not alter the mortality rate of the beetle. Size and sex of the beetle, species of resin, and temperature of the chamber did alter the mortality rate.

A project has been undertaken to investigate the resistance of pines to bark beetles. Work by Callaham (1953) indicated that resin might be toxic to bark beetles. In pursuing this possibility, the development of suitable techniques has been a major obstacle. Both the habits of the beetle and the nature of resin influenced the course of study and the methods. The beetle's habit of boring beneath the bark in the phloem and cambial layers of living trees suggested that if resins are toxic, they may function through contact, stomach, or fumigant action. Fumigant toxicity was chosen for the subject of research because the characteristics of fresh resin make it difficult to use in contact and stomach toxicity tests. The western pine beetle, *Dendroctonus brevicomis* Lec., was used most extensively in the early phases of the work reported here; after resolution of techniques the Jeffrey pine beetle, *D. jeffreyi* Hopk., was used for comparative purposes. This paper is concerned with the development of techniques.

Resin is essentially a supersaturation of a solid phase in a liquid phase; the solid is extracted commercially as rosin, the liquid as turpentine. Each phase is a complex mixture of compounds. The molecular structure of some of these, especially of rosin compounds, has not been fully determined. The fresh resin of the trees used in this work is difficult to maintain in its natural state. As resin flows from the tree it is a clear, colorless or amber-colored, viscous liquid. Within a few hours physical, and possibly chemical, changes start: the two phases begin to separate, resulting in the depositing of the solid phase as a crystallinelike material and formation of a supernatant, less-viscous liquid. If the liquid, or fresh resin, is not tightly contained, it begins to volatilize immediately.

At present resin is considered a secondary plant substance. It is a by-product of metabolism and serves no

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FIG. 1.—Technique and materials for collecting resin from pine.

role in the basic functions of growth and reproduction, though there has been a long-standing assumption that it serves a vital role in the protection of the tree from insects and disease.

This article presents the methods used for extracting resin from pine trees, for rearing beetles, and for construction and assembly of the chambers necessary to confine the beetles with the vapors of resin. Two techniques which were successfully used to determine the effect of various physical factors on the fumigant action of resin vapors to beetles are described and compared.

MATERIALS AND METHODS.—*Extraction of Resin.*—Resin was extracted from the trees by essentially the same method used by Callaham (1955). A circular-punch wound, $1\frac{1}{2}$ inches in diameter, was made through the bark and phloem and about $\frac{1}{4}$ inch into the sapwood. The plug of wood and bark was removed and a plastic cylinder was pushed into the hole (fig. 1). The cylinder was $2\frac{1}{2}$ inches long, with the outer end closed and a hole through the curved surface just inside the closed end. This hole was fitted with a 1-inch-long plastic tube with cork around it. After the cylinder was fitted into the wound, the hole was oriented downward and a 30-cc. shell vial was attached to the tube. Thus the resin flowed from the wound, into the plastic cylinder, down the hole, and into the vial. The shell vial was easily replaced without disturbing the rest of the collecting device.

Separation of the physical phases of some resins starts within an hour or two after the first flow of resin from the wound but decreases with continued flow. Therefore, by allowing the resin to flow from the wound for 2 to 4 hours, changing the shell vial, and using the second collection for experimentation, clear, unseparated resin was obtained for testing.

Rearing of Beetles.—The two species of beetles were reared from naturally infested material obtained from the forest when the insect had reached the late larval or the pupal stage. The two beetles differ in their developmental habits (Keen 1955, Eaton 1956), and therefore the infested material had to be handled differently. Since *D. brevicomis* matures in the corky layers of the bark of ponderosa pine, *Pinus ponderosa* Laws., it was necessary to remove only the bark from the infested tree. *D. jeffreyi*, however, matures in the phloem and cambial layers of Jeffrey pine, *P. jeffreyi* Grev. and Balf., and separation of the bark from the wood disrupts development of the insect. For this beetle it was necessary to obtain a segment of wood and bark together. This was accomplished by felling and sectioning the infested tree and then slabbing off conveniently sized segments with wood and bark intact.

The infested material was gathered at the proper time and held in a coldroom at 35° F., never for more than 4 months. When ready for use, the infested material was transferred to a screened, roofed, outdoor insectary where the beetles were allowed to complete their development and emerge under normal diurnal summer conditions, occurring at the Institute of Forest Genetics near Placerville, in the Central Sierra Nevada. As the beetles emerged, they were collected singly in #000 gelatin capsules to prevent injury to one another and to facilitate subsequent handling. Collections, which were simplified because the beetles habitually went to the sides of the insectary after emerging, were usually made in the morning and afternoon, and the beetles were held at 35° F. until needed.

Fumigation Chamber.—The two types of chambers used in the fumigation testing work were a standard 30-cc. test tube stoppered with cork, and a 4-ounce (150 cc.) screw-cap jar with a disk of teflon plastic serving as a gasket in the cap (fig. 2h).

Other types of chambers were tried but found to be unsuitable. Stoppering the test tube with neoprene or with polyethylene-wrapped corks was less suitable than cork alone. The materials either absorbed the resin vapors or failed to stopper the tube adequately. Wide-mouth weighing bottles with ground glass unions, with or without sealing compound, were also unable to contain the vapors adequately.

Container for beetles.—Each beetle was confined to a small cell within the fumigation chamber. Because of the difference in size of the two beetles, two types of cells were necessary. For *D. brevicomis* the cell was a 1-inch length of 5-mm. i.d. glass tubing. Four of these lengths were tied into a bundle with nylon thread (fig. 2a); a disk of 32-mesh lumite screening, which was tied to one end of the bundle, served as a base for the cells (fig. 2b). The other end of the cell was covered with lumite at the time the chamber was assembled. For *D. jeffreyi* the cell was a $\frac{1}{4}$-dram vial (fig. 2f) which was plugged with a disk of lumite (fig. 2j).

Assembly of chamber.—Only collected beetles held in cold storage for less than 32 hours were used in experiments, except where the factor of period of cold storage was being studied. Just before a test was assembled, all beetles were equitably distributed among the replicates by size and by age of storage.

Experiments with *D. brevicomis* in a 30-cc. test-tube chamber were assembled as follows. A replicate of beetles was taken at random from those assigned to the test. Each beetle was transferred from a gelatin capsule into

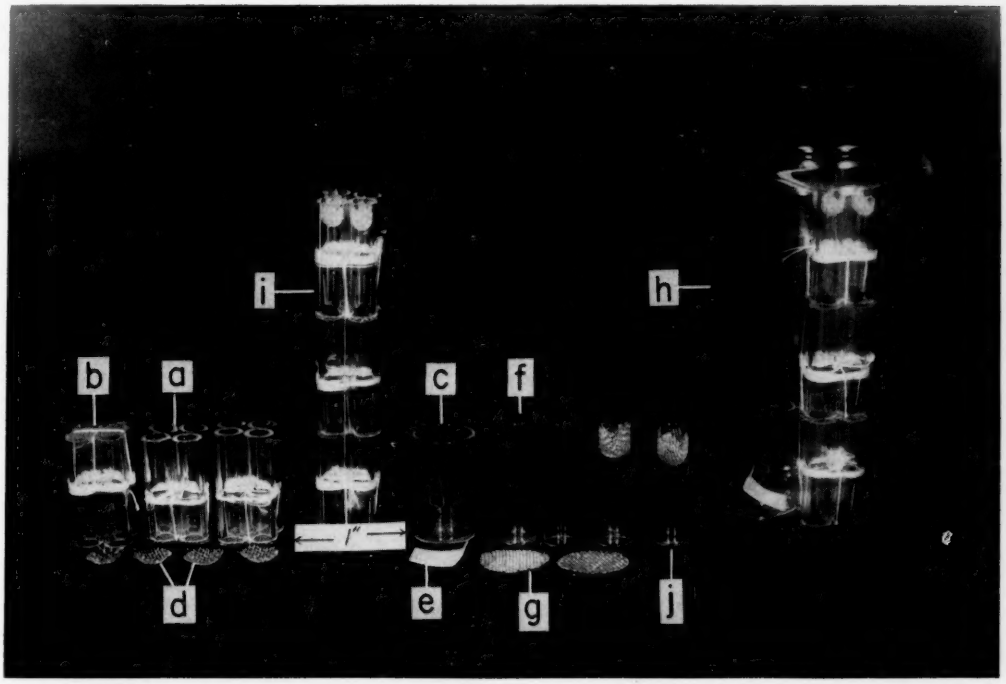

FIG. 2.—Four-ounce jar fumigation chamber. (a) Bundle of 4 cells with (b) base of lumite mesh; (c) resin vial, (d) small lumite plugs, (e) teflon square, (f) ¼-dram vial, (g) lumite disk for ¼-dram vial plug, (h) assembled jar, (i) stack of 3 bundles of cells, (j) plugged vial.

a cell of the bundle. Since the beetles had been at 35° F., they were inactive while being transferred. As soon as the beetles of a replicate were in cells, the two or three bundles were placed in a test tube. A small glass cylinder was placed in the bottom of the test tube to insure that all stacks of cell bundles were at the same height in the tube. As each bundle was placed in the tube, a disk of lumite (fig. 2g) was placed on top of it. This disk, held in place by the bundle above it, made each cell completely enclosed. The disk of the top bundle was temporarily held in place by a glass cylinder which was later replaced with a vial of resin. The desired amount of resin was pipetted into a small resin vial (fig. 2c) which was immediately placed in the test tube on top of the stack of bundles. The tube was tightly stoppered and placed under the conditions of the test.

The assembly of the 4-ounce jar fumigation chamber was quite similar to that of the 30-cc. test-tube chamber. Three bundles of cells were arranged in a stack and bound together with a single thread of nylon (fig. 2i). Since the stack was tightly bound, the bottom of one bundle acted as a top for the cells of the bundle beneath it, and no additional lumite disks were needed. The cells of the top bundle were plugged with small pieces of lumite mesh. A stack of three bundles, constituting a replicate of 12 beetles, was placed in a jar. Resin was then apportioned into the small resin vials. Macro-amounts, greater than 0.01 ml., were apportioned with a pipette; micro-amounts, less than 0.01 ml., were apportioned by turning a micrometer shaft against the plunger of a hypodermic syringe. The micro-amount of resin which formed as a droplet on the end of the needle was picked up with a ¼-inch square of teflon (fig. 2e) and immediately dropped into a resin vial. The resin vials were quickly put into the 4-ounce jars containing beetles and the jars were capped (fig. 2h). Usually less than 5 seconds elapsed between apportionment of the resin and placement of the vial into the 4-ounce jar. To determine the amount of vapor produced by each volume of resin used in a test, additional samples were placed in jars without beetles; the loss of weight of these samples, as measured by a series of analytical weighings, was considered the weight of the vapor in the jar. Since the loss of weight was quite consistent for different samples of the same approximate volume of resin, it is assumed that weighed samples of resin represent the similar samples used with beetles.

For experiments with *D. jeffreyi*, each beetle was transferred to a ¼-dram vial which was then plugged with a disk of lumite (fig. 2j). This species of beetle was not used with the 30-cc. test tube chamber.

The number of replicates in a test varied; there were usually 5 or 6 for a given set of conditions, and no test had less than 4. There were 8 to 12 *D. brevicomis* and 5 *D. jeffreyi* in each replicate. Periodic, usually daily, observations were made of beetle mortality; a beetle was considered dead if it failed to respond to agitation. In most tests with the 30-cc. test tube, the beetles were confined in the chamber for the duration of the test. With the 4-ounce jar, they were removed at designated intervals and kept in the cells in the atmosphere of the

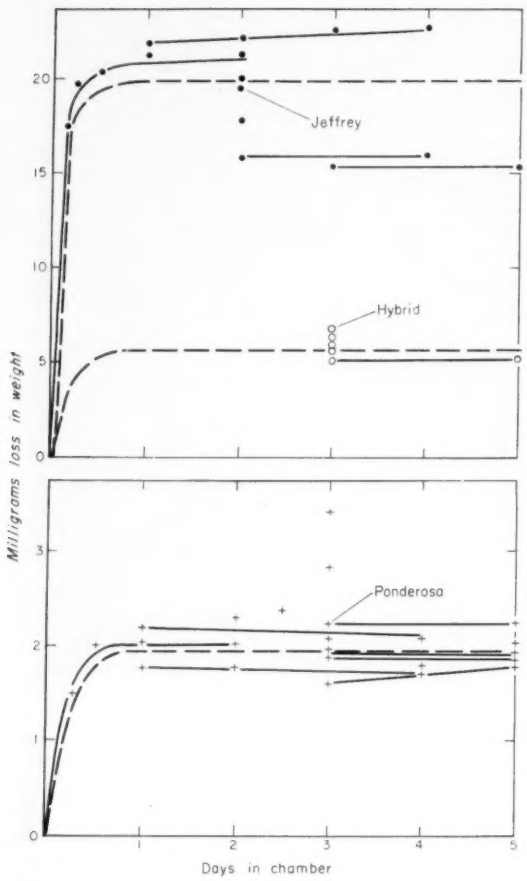

FIG. 3.—Weight loss of three pine resins in 150-cc. volume at 70° ± 2° F. Dashed line is generalized curve. Solid lines connect measurements of different sets of samples of the same extraction of resin. Other points were obtained from other tests.

room at the temperature of the test. Post-mortem determinations were made of sex and size of each beetle to see if such factors influenced the results.

RESULTS.—The tests reported here were conducted (1) to determine suitability of the methods and materials, (2) to determine some of the properties of resins and the physical factors which might affect the action of resin vapors on adult bark beetles, and (3) to compare results obtained by the two types of chambers.

Absorption of Resin Vapors.—Before extensive tests were conducted, preliminary investigations were made of the absorption of resin vapors by the materials used in the fumigation chamber. Whenever accurate determinations are to be made of the amount of vapor in a chamber, the materials in the chamber must not absorb any vapor.

Absorption was determined by confining samples of the various materials in the 4-ounce jar in which there was a saturated atmosphere of ponderosa or Jeffrey pine resin vapor. Sets of samples were removed and reweighed at intervals. Any increase in weight above similar untreated checks was considered the weight of absorbed vapor. Cork, neoprene, and polyethylene increased in weight in the resin vapor atmosphere; all samples except cork were quite uniform in their change in weight. Teflon, lumite, and nylon did not change (table 1). Thus, the former three materials were ruled out for use with resin vapors and the latter three were permitted.

Rate of Vaporization and Vapor Saturation.—To conduct tests properly and handle resin effectively, it was necessary to determine how rapidly vapors escaped from confined resin and also how much vapor was required to saturate the confined atmosphere. Weighed samples of ponderosa and Jeffrey pine resin were placed in 4-ounce jars, and at designated intervals sets of samples were removed and reweighed. There were four jars for each time interval. The values obtained for a given set were very constant and are connected by the thin, curved line in figure 3. Judging from generalized curves of the loss of weight of each of the three resins at 70° ± 2° F. (heavy broken lines, fig. 3), saturation was reached by the end of the first day. It is likewise apparent that the weight of ponderosa pine resin vapor necessary to produce saturation is about one-tenth that of Jeffrey pine resin. The resin of the Jeffrey-ponderosa pine hybrid is intermediate between that of the two parent trees. Variations in the data during the course of the work could have been caused by seasonal changes in the resins or by inconsistencies in technique, though special care was taken to avoid the latter. To eliminate possible tree-to-tree variation, one tree was used for all ponderosa resin and one for all hybrid resin. Two trees with the same parentage were used as the source of the Jeffrey resin.

Effect of Physical Factors on Fumigant Action.—In attempting to draw sound conclusions from this type of experimentation, it is usually quite important to control or account for the major physical factors. Several tests were conducted to determine the effect of various factors on the mortality rate of *D. brevicomis* in the 30-cc. test tube chamber. The 30-cc. test tube was used in all tests except where volume was the variable factor; in this test the appropriate sized test tube was used. All tests were made under normal fluctuating room temperature and

Table 1.—Absorption of ponderosa and Jeffrey pine resin vapors by various materials after different periods of confinement in a saturated atmosphere.

RESIN	MATERIAL	APPROXIMATE WEIGHT OF MATERIAL (MG.)	WEIGHT INCREASE (MG.) AT DAYS OF CONFINEMENT SHOWN[a]		
			1	2	3
Ponderosa	Cork	250	2.2	0.2	0.6
	Neoprene	2,200	11.8	22.1	68.1
	Polyethylene	50	0.6	1.3	1.9
	Teflon	150	0.0	0.0	0.0
	Lumite	150	0.0	0.0	0.0
	Glass	180	0.0	0.0	0.0
	Nylon	170	—	0.0	—
Jeffrey	Lumite	350	0.0	0.0	0.0
	Teflon	120	0.0	0.0	0.2
	Nylon	160	—	0.0	—

[a] Each measurement represents the average of five samples.

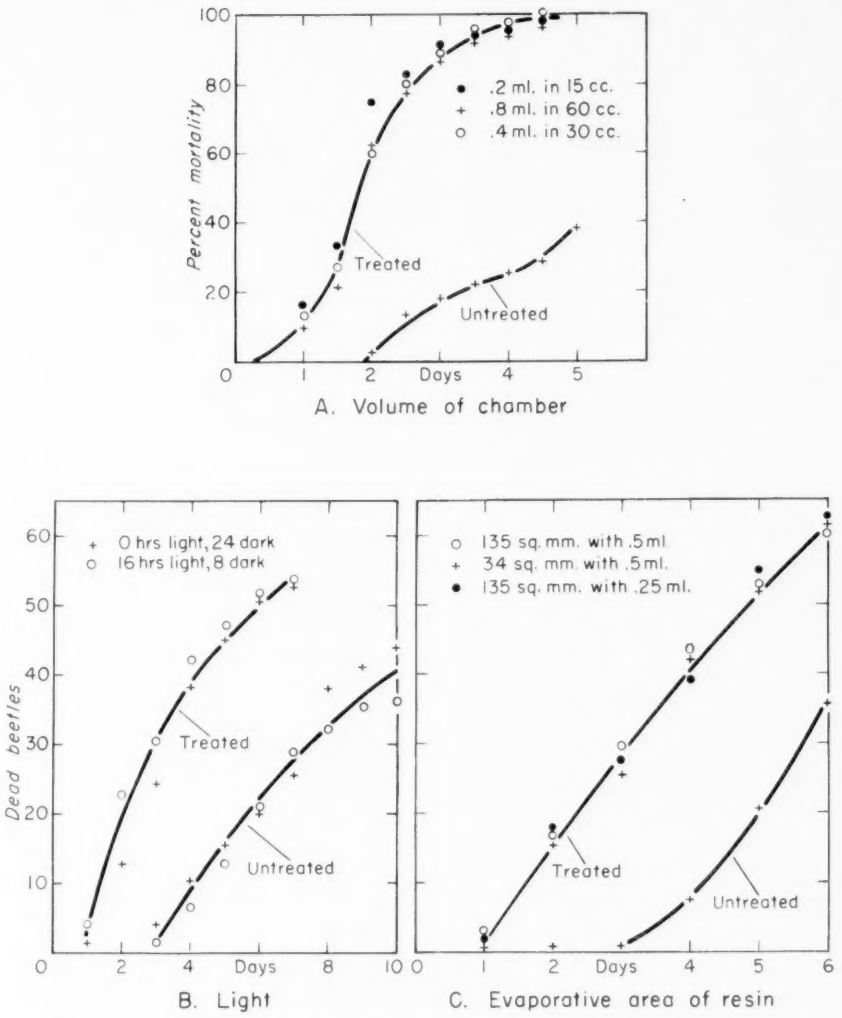

FIG. 4.—Factors which did not affect reaction of *Dendroctonus brevicomis* to saturated vapors of ponderosa pine resin from 70° to 90° F.

light, except where these two factors were controlled as noted. No effort was made to determine the amount of vapor in the tube; instead, dosage was determined simply on the basis of the volume of resin. Actually, all dosages were sufficient to saturate the chamber.

Mortality rate was not affected by variations in the volume of the chamber between 15 cc. and 60 cc., by the evaporative area of resin, or by the presence or absence of light. Variations in these factors did not alter the position of the mortality-rate curve (fig. 4).

Mortality rate was affected by the length of refrigeration of the beetle, by the size and sex of the beetle, by the temperature, and by the species of resin. Variations in these five factors noticeably altered the position of the mortality-rate curve (figures 5 and 6A).

The approximate physical conditions represented in figure 6A were then repeated. No beetles were used, and the samples of resins were removed and weighed at intervals to determine the rate of loss of weight of the resins in the test tube; this loss of weight was assumed to be the weight of the resin vapor in the tube. The results reveal an abrupt change at 1 day in the slope of the line representing the rate of loss of weight of resin (fig. 6B). This indicates that within 1 day the resin vapors were at or near saturation. Furthermore, the chart shows a continued upward trend of the weight-loss line after the first day, indicating continued loss of vapor either by absorption by the cork or by leakage through it. The use of a teflon-sealed screw cap jar eliminated this error.

These tests showed the necessity for controlling the

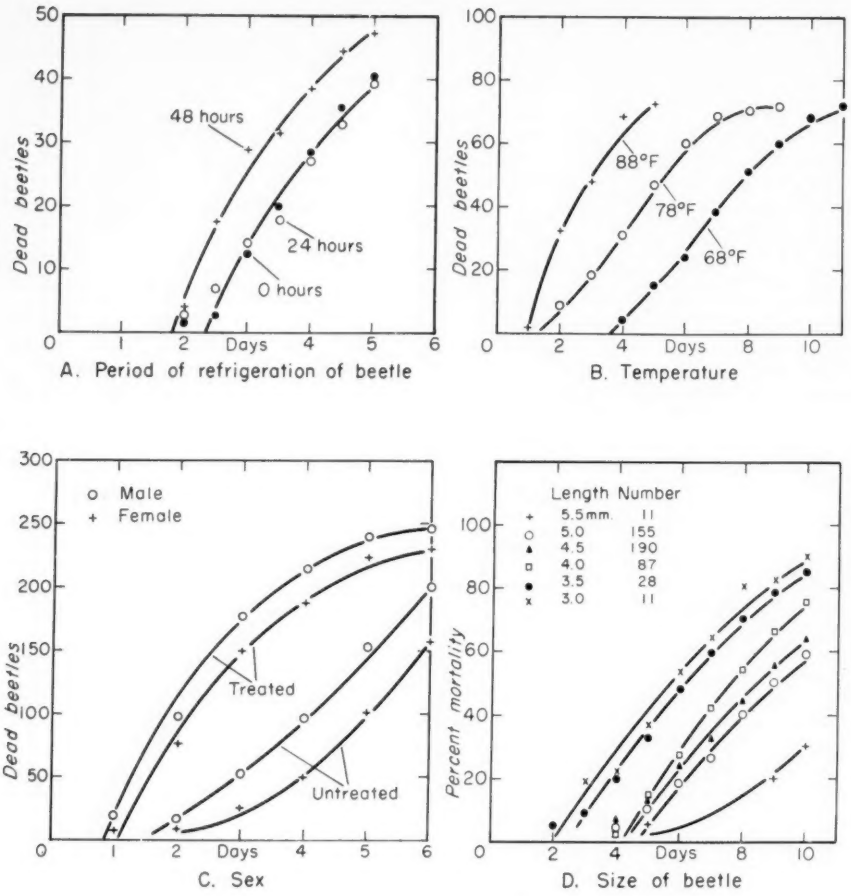

FIG. 5.—*A*, *B*, and *C* are factors which affect the reaction of *Dendroctonus brevicomis* to saturated vapors of ponderosa pine resin at temperatures from 70° to 90° F.; *D* is effect of size of beetle on the rate of natural mortality.

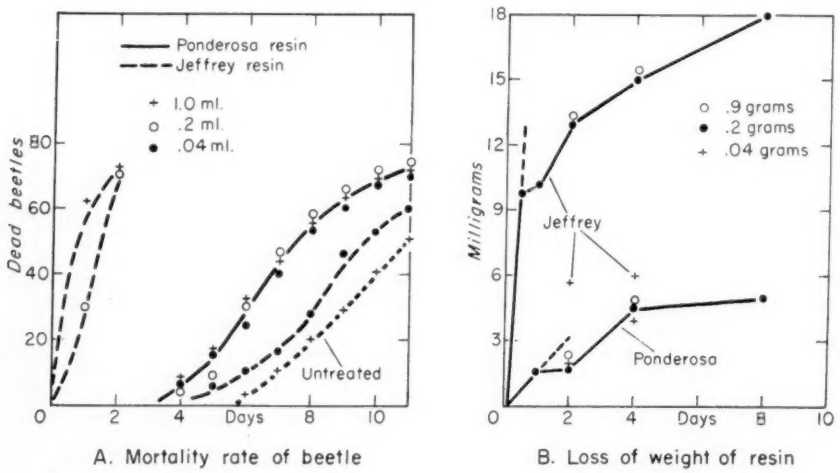

FIG. 6.—*A*, mortality of *Dendroctonus brevicomis* in 30-cc. test tube chamber at 68° F. with varying volumetric dosages of ponderosa and Jeffrey pine resin; *B*, loss of weight of resin samples in 30-cc. test tube chamber at 68° F.

temperature and the time of refrigeration of beetles and for equitably distributing the beetles by size. Distribution by size also in a large measure overcame the factor of sex, since sex is correlated with size, females being larger than males.

Comparison of Two Types of Chambers.—The 30-cc. test tube and the 4-ounce jar fumigation chambers were compared. In these tests the beetle-to-volume and resin-to-volume ratios were equal in the two types of chambers. Ponderosa pine resin was used, and its vapor concentration was expressed only as saturation or subsaturation because accurate determination could not be made in the cork-stoppered test tube. The exposure periods used were 3 and 5 days, at which times the cells were removed from the chambers. In the 30-cc. test tube, the resin vapor produced a distinct toxic effect, and the rate of natural mortality in a nonresinous atmosphere in such test tubes exceeded that in the 4-ounce jar (table 2). Earlier tests had demonstrated that the rate of natural mortality was the same in or out of the 4-ounce jar. Thus, it would appear that some condition was created in the 30-cc. test tube that increased the rate of mortality in both checks and treatments. However, since the effect of the condition was somewhat proportional with various materials and conditions, the 30-cc. test tube still may be used for rough comparisons of materials and conditions affecting mortality.

The 4-ounce jar was selected as the test chamber for conducting fumigation experiments because (1) it completely contained the resinous vapors, (2) it permitted accurate determinations of the vapor in the jar, and (3) it caused no increase in the rate of natural mortality. An extensive series of tests were made with the 4-ounce jar to compare the reaction of the two species of beetles in saturated and subsaturated vapors of ponderosa and Jeffrey pine and of the hybrid between these two pines (Smith 1961).

Table 2.—Mortality of *D. brevicomis* in vaporous atmospheres of ponderosa pine resin in two types of chambers.

Chamber	Concentration of Vapor[a]	Total Beetles Used	Mortality (No.) After Exposure of—	
			3 Days	5 Days
Jar (4-ounce)	Saturation	60	1	10
	Saturation	60	2	10
	Subsaturation	60	2	6
	Untreated	60	2	4
Test tube (30-cc.)	Saturation	60	15	26
	Saturation	60	16	25
	Subsaturation	60	3	11
	Untreated	60	0	12

[a] Expressed only as saturation and subsaturation because vapor concentration could not be determined accurately in the cork-stoppered test tube.

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The Fumigant Toxicity of Three Pine Resins to *Dendroctonus brevicomis* and *D. jeffreyi*[1]

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ABSTRACT

The toxicity of pine resin vapors to adult bark beetles was selected as one means of investigating the resistance of pines to these insects. When adults of *Dendroctonus brevicomis* Lec. were confined to a saturated atmosphere in a 30-cc. test tube, mortality rates differed significantly between species of resins and between resin and turpentine (the liquid commercial derivative of resin).

In a 150-cc. screw-cap jar fumigation chamber, *D. brevicomis* and *D. jeffreyi* Hopk. were able to tolerate the volatile fraction of their natural host resin, respectively *Pinus ponderosa* Laws. and *P. jeffreyi* Grev. and Balf.; but neither beetle was able to tolerate the vapors from the other's host resin. With increased time of exposure, neither beetle was able to tolerate the saturated vapors of resin from a *P. jeffreyi*×*ponderosa* hybrid. This led to the hypothesis that bark beetles can tolerate the resin vapors of their hosts but cannot tolerate that of a nonhost.

Recently there has been increasing interest in the role of secondary plant substances in insect host selection, specificity, and susceptibility. Despite some debate about the meanings of these three terms, essentially the basic contention is that secondary plant substances determine if, when, and how successfully an insect attacks a plant. In fact Fraenkel (1959) suggests that ". . . the food specificity of insects is based solely on the presence or absence of these odd (secondary) compounds in plants which serve as repellents to insects (or other animals) in general and as attractants to those few which feed on each plant species."

Does resin play a significant role in the success or failure of a bark-beetle attack on a pine tree? Mirov (1959) mentions the wide assortment of volatile substances in pine resins. These could act in the manner

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proposed by Fraenkel. Resin is considered a secondary plant substance—essentially a by-product of metabolism that plays no role in growth and reproduction. There has been a long-standing assumption, with little or no proof, that resin protects the tree from insects and disease. Nevertheless, resin-producing trees have a complement of insects and diseases which is not unlike that of non-resin-producing trees.

In an attempt to answer the question, J. M. Miller (1950) started research aimed at incorporating insect resistance into the forest tree improvement program and at gaining a better understanding of the fundamental relationships which exist between bark beetles and pine trees. Actually there had been some related work in California between 1927 and 1933. These studies are summarized by Miller & Keen (1960). As a continuation of Miller's early exploratory studies, Callaham (1953) conducted forced-attack studies in which beetles, or bark from which they would soon emerge, were caged around the basal 8 feet to 12 feet of various species or conditions of trees. The beetles were thus forced to attack one particular tree. The course of these attacks was observed and an evaluation was made of the behavior of the beetles and of the extent and success of their attacks. Beetles entered all pines which they were forced to attack, but they were less successful and were less typical in their activity on trees that were not their host under natural forest conditions.

The results, though possibly quite indicative, were sometimes difficult to interpret because of the lack of suitable data. Callaham (1955) then made a rather extensive study of the quantity of resin produced by ponderosa pine and the factors that affect it. Though he was unable to correlate total resin flow with the tree risk (susceptibility) classification of Salman & Bongberg (1942), he did find that trees classified as more susceptible to bark-beetle attack produced very little resin after the third day of wounding. Trees classified as less susceptible continued to produce resin after the third day. This flow after the third day could be "the straw that breaks the beetle's back" and causes the attack to fail.

Because these early studies did not make it possible to pin-point confidently the factors which may cause a bark-beetle attack to succeed or to fail, the problem was reanalyzed to determine the future course of the work.

Painter (1951) contends that studies of plant resistance to insects should be carried out with the plant materials that are intimately associated with the insect. Gross fresh resin is a plant material associated with pine bark beetles. Thus the decision was to work with gross fresh resin and not derived products of resin until the gross material could be proven toxic. Painter classifies the phenomena of resistance into three mechanisms: preference, antibiosis, and tolerance. This classification was followed in deciding the manner in which resin could cause a pine tree to be susceptible or resistant to bark-beetle attack.

Toxicity, or antibiosis, is suggested by repeated references by those working closely with bark beetles to their attacks being "pitched out," and by the work of Miller and Callaham. However, more adequate quantitative data were needed to substantiate the contention that resin can cause the death of bark beetles. Preference is suggested by frequent reference to the selection by bark beetles of certain conditions of trees. For these reasons, both toxicity and preference were selected as the subjects for investigation. Though both are considered equally worthy of study, research on preference has progressed more slowly than research on toxicity because of the lack of adequate technique.

The approach to the investigation of toxicity was shifted from a broad scope in which the beetle was confronted with a living tree, with its multitude of changing and uncontrollable factors, to a narrower scope in which the beetle was confronted only with resin. By this shift, data were obtained which seem more suitable to analysis and interpretation. This paper presents some of the more significant results of research on the fumigant toxicity of pine resins to bark beetles (Smith 1959). The techniques employed will be dealt with rather briefly since they are the subject of a separate paper (Smith 1961).

THE PROBLEM OF TOXICITY.—Before experimentation on toxicity of pine resins to bark beetles was begun, an effort was made to narrow the area of research to the most likely and amenable subjects by considering the habits of the beetles. This was of little help because their habit of boring beneath the bark of living trees and feeding on resin-impregnated tissue suggests that resin, if it is toxic at all, could be toxic through contact, stomach, or fumigant action. However, preliminary work indicated that the physical properties of resin had to be considered in determining the course of the work. Resin is a difficult material to handle and maintain in its natural state. Within a few hours after its extraction from many species of pine trees, resin—a clear, colorless or amber-colored, viscous liquid—begins to separate into a solid and a liquid phase. If the latter is not completely contained, it evaporates. These properties of resin make stomach and contact toxicity studies difficult, since the decision was to work with fresh resin as it is encountered by the beetle boring in the cambial layer beneath the bark. Therefore, the fumigant action of fresh resin vapors was chosen as the first subject for research.

In the early phases of the work *Dendroctonus brevicomis* Lec., the western pine beetle, was selected as the test insect not only because it is the most destructive pest of ponderosa pine, *Pinus ponderosa* Laws., but also because it has a very limited number of hosts; Coulter pine, *P. coulteri* D. Don, is its only other recognized host. As soon as techniques were resolved, the Jeffrey pine beetle, *D. jeffreyi* Hopk., was included in the testing program. This beetle is found only on Jeffrey pine, *P. jeffreyi* Grev. & Balf. Thus neither beetle is found on the other's host. Though ponderosa and Jeffrey pines are supposedly very close taxonomically and though natural hybrids occur in the forest, their resins are quite different (Mirov 1929). Therefore one segment of the problem which received major attention was the reaction of the two species of beetles to the resin of ponderosa pine, Jeffrey pine, and the Jeffrey×ponderosa pine hybrid.

MATERIALS AND METHODS.—Essentially the procedure was to expose mature beetles, placed in individual cells, to resin vapors in a small glass fumigation chamber (Smith 1961). Two types of chambers were used: a cork-stoppered 30-cc. test tube or a 4-ounce widemouth screw-cap jar with plastic teflon gasket in the cap. Fresh, un-

crystallized resin was extracted from the tree with a micro-tap device and was then apportioned by volume into small vials. In one test a processed resin derivative, as well as natural resin, was used. One of the small vials was placed in each chamber and the chamber was securely closed. In the 30-cc. test tube the resin dosage was determined simply on the basis of the volume of resin. In the 4-ounce jar the dosage was the actual weight of vapor in the chamber as determined by a series of weighings of samples of resin.

Beetles were collected as they emerged from naturally infested material gathered in the forest and held in a screened insectary. The beetles, all of approximately the same age, were equitably distributed by size among the various conditions of the particular test. Beetles were held in the resinous vapor atmosphere of the 4-ounce jar for specified times and then removed and observed; those in the 30-cc. test tube were held in the tube for the duration of the test. All tests were maintained in the dark at 70° ± 2° F. The number of replicates varied, though five or six usually were used for each condition of a test. Each replicate consisted of 8 or 12 *D. brevicomis* or 5 *D. jeffreyi*. Observations were made at daily or 2-day intervals. Beetles were considered dead if they did not move when agitated. Post mortem determinations were made of the size and sex of the beetles. If considered necessary, a t-test was made of the mortality data and a 90% confidence level was used as a criterion for significance.

RESULTS.—The first studies showed that the 30-cc. fumigation chamber had certain deficiencies (Smith 1961). It did not permit determination of the actual amount of vapor in the chamber; the cork either absorbed the resin vapors or let some escape. Also, a condition was created in the 30-cc. chamber that caused an increase in the rate of mortality of the beetles in both treated and untreated atmospheres. However, since the effect of the condition was somewhat proportional in treatments and checks, the results of tests with the 30-cc. test tube are considered suitable for rough comparison of the mortality rate of beetles with three kinds of resin and of mortality with resin and derivatives.

To compare the mortality rate of *D. brevicomis* in a saturated atmosphere of the three pine resin vapors, two volumes of resin, 1.0 ml. and 0.1 ml., were used with 6 replicates of 12 beetles each for each condition of the test. These two volumes were more than sufficient to produce a saturation of resin vapors in the 30-cc. chamber.

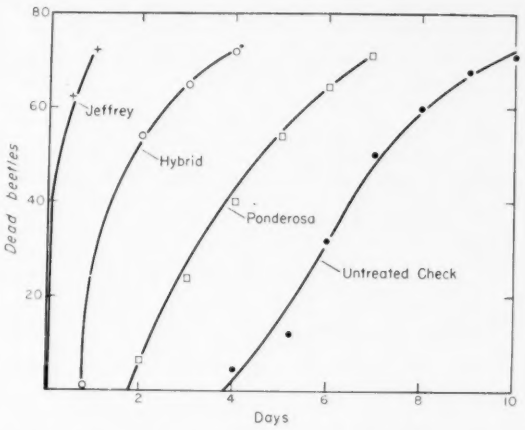

FIG. 1.—Effect on *Dendroctonus brevicomis* of confined saturated vapors of ponderosa, Jeffrey, and hybrid pine resin in 30-cc. test tube.

All three treatments and the untreated check are significantly different from each other in the number of dead beetles on a given day (fig. 1). The mortality rate in hybrid resin vapor was significantly faster than in ponderosa resin vapors, and the rate in Jeffrey resin vapor was significantly faster than in the hybrid. It will be noted that saturated resin vapors of ponderosa pine caused a significant increase in the rate of mortality over that of the untreated checks. A lack of such an increase in the 4-ounce chamber led to the conclusion that a deleterious condition was created in the 30-cc. test tube.

The effects of resin and its derivative were compared by using saturated vapors (0.5 ml. in 30-cc. chamber) of (a) fresh resin of ponderosa pine, (b) the supernatant liquid obtained by natural crystallization at room temperature, and (c) turpentine prepared by fractionation at 165° C. This experiment was made because some workers feel that turpentine exists as such in pine resin and therefore tests of resin toxicity to bark beetles could be greatly simplified by working with turpentine.

There were eight beetles per replicate and 10 replicates

Table 1.—Cumulative mortality of *D. brevicomis* confined with the vapors of ponderosa pine resin and its derivatives.

Material	Exposure of Resin (hrs.)	Weight[a] Loss (mg.)	Total Beetles Used	Total Beetles Dead on Days After Start of Test								
				1	2	3	4	5	6	7	8	9
Resin	24	2.5	40	0	2	4	5	6	10	21	28	30
	48	2.9	40	0	3	5	8	15	21	26	31	35
Supernatant liquid	24	2.6	40	0	0	2	3	6	8	18	27	38
	48	3.7	40	0	2	3	7	11	17	21	27	33
Turpentine	24	6.2	40	2	3	8	14	20	33	37	39	39
	48	7.7	40	—	11	22	27	34	38	38	39	40
Untreated	24	0.0	40	0	0	2	3	3	5	9	11	14
	48	0.0	40	0	0	0	1	3	7	11	13	16

[a] Each an average of 5 samples, no correction for absorption of vapor by cork.

Table 2.—Mortality of two bark beetles with different periods of exposures to vapors of three pine resins in a 150-cc. chamber.

Resin	Test Number	Vapor Weight (mg.)	No. Beetles Used for Each Exposure	Per Cent Mortality When Exposure Was		
				3 Days	5 Days	7 Days
		Dendroctonus brevicomis				
Ponderosa	1	Check	48	—	—	25
		0.5	48	—	—	17
		0.9	48	—	—	25
		1.8[a]	48	—	—	15
	2	Check	108	4	—	—
		0.9	108	1	—	—
		1.8[a]	108	9	—	—
	3	Check	72	6	14	49
		1.2	72	8	17	36
		1.7[a]	72	7	18	39
		1.8[a]	72	6	19	46
Jeffrey	1	Check	108	3	—	—
		0.5	108	6	—	—
		1.0	108	7	—	—
		1.9	108	6	—	—
		3.8	108	4	—	—
	2	Check	72	3	18	46
		2.7	72	4	13	25
		3.8	72	0	15	28
		5.3	72	10	21	40
		22.4[a]	72	100*	100*	100*
	3	Check	48	0	13	—
		2.9	48	4	17	—
		6.0	48	8	29*	—
		11.3	48	63*	100*	—
		17.7[a]	48	100*	100*	—
Hybrid	1	Check	48	6	23	25
		0.9	48	4	15	29
		5.4[a]	48	6	33*	52*
		Dendroctonus jeffreyi				
Ponderosa	1	Check	30	0	—	—
		1.2	30	0	—	—
		2.0[a]	30	13	—	—
	2	Check	20	5	50	85
		1.1	20	0	30	90
		2.4[a]	20	0	85*	100
Jeffrey	1	Check	30	0	—	—
		1.2	30	0	—	—
		19.8[a]	30	0	—	—
	2	Check	20	5	50	85
		0.9	20	10	55	80
		19.9[a]	20	10	50	100
Hybrid	1	Check	15	13	13	87
		0.8	15	20	27	87
		6.6[a]	15	27	73*	93

[a] Vapor at saturation.
* Significantly different from check at 90% confidence level or greater.
—, No tests made.

for each of the three materials and an untreated check. Resin vials were removed from half of the replicates at the end of 24 hours and from the remaining half at 48 hours. The resin vials, located in the top of the test tube, were removed quickly and carefully and the test tube was immediately restoppered with a minimum of disturbance to the fumigant atmosphere. Weighings were made to determine the weight of the vapors which had accumulated in the fumigation chamber. Unfortunately some of the vapors were absorbed by the cork stopper or escaped through it. Nevertheless, for rough comparisons, the data are considered quite adequate because the atmosphere in the tube was saturated with the particular resinous material.

The losses in weight for each of the three materials (table 1) show that fresh resin—with losses of 2.5 and 2.9 milligrams—is very similar to the supernatant liquid—with 2.6 and 3.7 milligrams. However, turpentine—with losses of 6.2 and 7.7 milligrams—is distinctly different from these two natural nonprocessed materials. A distinct difference is also apparent in the rate of mortality of beetles caused by these three materials. The number of dead beetles was consistently lower in saturated vapor of resin and supernatant liquid than in turpentine vapor.

Thus quite definitely, turpentine and the supernatant liquid of resin are not synonymous either in their physical properties, as indicated by the loss of weight in a confined atmosphere, or in the biological action of their volatile forms to the western pine beetle. The obvious conclusion is that some of the basic constituents of natural resin are changed or lost by the process of heat fractionation.

A large series of tests was made over a period of 2 months to compare the mortality of *D. brevicomis* and *D. jeffreyi* in the vapors of the three resins in the 4-ounce jar fumigation chamber. Both saturated and subsaturated vapor concentrations were used. The periods of confinements were 3, 5, and 7 days, though in some tests fewer than these three periods were used; *i.e.*, the number of replicates was sufficient for observation at only one or two of these periods. The results are given in table 2; the untreated check is given for each test because the tests were conducted with different broods of beetles at different times during the summer. The data were analyzed by the "t" test comparing each value with its corresponding untreated check.

There is admittedly a need for larger samples of the Jeffrey pine beetle and for more extensive and comprehensive testing before well-substantiated conclusions can be drawn. Nevertheless there is a general picture in the data which does justify some consideration. The western pine beetle showed no significant mortality with any dosage of resin vapor of its recognized host, ponderosa pine (table 2). Resin vapors of Jeffrey pine, a nonhost tree, did cause significant mortality at both saturation and at a subsaturated dosage of 6.6 milligrams per 150 cc. volume. Significant mortality occurred with hybrid resin vapors, but only at saturation for exposures of 5 days or more.

Likewise with the Jeffrey pine beetle the resin vapors of Jeffrey pine, its recognized host, caused no significant mortality; but significant mortality was caused by the resin vapors of ponderosa pine, a nonhost tree, and by the resin vapors of hybrid pine. The rate of natural mortality of the Jeffrey pine beetle was too rapid to permit an analysis of the data obtained with a 7-day exposure.

Therefore it appears that each beetle can tolerate the resin vapor of its own host tree but cannot tolerate that of the other's host tree. Neither beetle can tolerate saturated concentrations of resin vapors of hybrid resin for an exposure of 5 days or more. These results present the possibility of resin, a secondary plant substance, acting in the manner proposed by Fraenkel; that is, determining whether a plant is the host of an insect.

CONCLUSIONS.—In a 30-cc. test tube fumigation chamber containing saturated vapors of certain pine resins, the mortality rate of the western pine beetle was (a) fastest in vapors of Jeffrey pine, (b) slowest in those of ponderosa pine, and (c) intermediate in those of the Jeffrey×ponderosa hybrid. The mortality rate was faster in saturated ponderosa pine resin vapors than in an untreated atmosphere.

With the same 30-cc. test tube procedure the turpentine of ponderosa pine resin was much more toxic to the beetle than fresh resin or its supernatant liquid. Turpentine also has a greater vapor saturation point. It therefore seems likely that some change takes place in the basic constituents of the natural compounds in resin during the fractionation process which produces turpentine.

Because of an artifact which limited the use of the 30-cc. chamber, it was necessary to use a 4-ounce jar chamber to obtain more precise data. In this type of chamber the western pine beetle showed: (a) no toxic reaction to ponderosa pine resin; (b) toxic reaction to Jeffrey pine resin at an above 50% vapor saturation but no toxic reaction below a 50% vapor saturation concentration; (c) toxic reaction to hybrid resin at saturation but no toxic reaction below saturation. On the other hand the Jeffrey pine beetle showed (a) no toxic reaction to Jeffrey pine resin; (b) toxic reaction to ponderosa pine resin only at saturation; and (c) toxic reaction to hybrid pine resin only at saturation with prolonged periods of exposure.

Therefore it would appear that resin and the host relationship of bark beetles may be closely associated and that a measure of host specificity or host susceptibility may be gained through the use of a simple test of fumigant toxicity to the beetle by resin vapors.

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Seasonal Losses in Rangeland Vegetation Due to Grasshoppers[1]

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ABSTRACT

Livestock-free grazing areas were used for studies of grasshopper damage on Montana rangelands. Half of each study area was sprayed with an insecticide at the beginning of the season to eliminate nearly all grasshoppers. The grasshopper population remaining on the unsprayed portion of the area was assessed by the use of cages that were placed over representative areas at night. Within both the sprayed and unsprayed portions of an area, sites were selected which appeared comparable from the standpoint of both the composition and abundance of the vegetation. Quadrats were established at these sites in which production was measured by clipping the vegetation at ground level, air-dried, and weighed. A comparison of the yield from an area without grasshoppers was thus made with that from an area on which grasshoppers fed.

Little correlation was found between numbers of grasshoppers per unit area and the loss of vegetation. This may be accounted for by the differences in feeding habits of the various species comprising any particular grasshopper population.

Further evaluation of the data from the various studies has proved more difficult. Problems have arisen primarily because of the nonrandom distribution of both grasshoppers and vegetation even where so-called uniform types of vegetation were considered. Difficulties in interpretation have also been encountered by the lack of knowledge concerning the phenomena of plant growth as shown by a study of two comparable areas during 1950–1959. These areas were subjected to grazing by livestock during the winter months only and the numbers of grasshoppers remained much below 1 per square yard during the period. Differences in yield of vegetation from the two areas were measured and it was found that considerable loss in forage may take place during a season without grasshoppers being present.

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Forage protection is the only sound basis upon which recommendations for the chemical control of grasshoppers infesting grazing lands can be made at the present time. Such recommendations, therefore, presuppose some knowledge of what one might expect in loss of forage from the presence of a particular grasshopper population. However, in most cases such knowledge is not available and control recommendations are then based upon past experience and the number of grasshoppers per unit area (usually the number per square yard). But in so doing, the assumption is apparently made that all grasshoppers are omnivorous and there seems to be disregard for existing differences in the feeding habits of the numerous species involved (Anderson & Wright 1952).

Various laboratory studies of the amount of food consumed by grasshoppers have been made. Although the amount eaten and the resulting losses in vegetation are not necessarily equivalent, such measurements have been used on occasion to estimate the amount of loss of forage in nature. For example, Montana entomologists (Anonymous 1926) reported that between 80° F. and 100° F. one adult ate 1 milligram of dry food per hour and as much as its own weight in green food every 15 to 18 hours. The importance of the effect of temperature on the amount eaten was pointed out in that at 100° F. an adult ate 2½ times as much per day as at 80° F. It was concluded from these studies that, since there were 418 hours considered to be favorable for grasshopper feeding between July 10 and October 1, 1919, and only 193 favorable hours during a similar period in 1918, each grasshopper during the 1919 season consumed 2.2 times as much as during the 1918 season. No mention was made of the species used in the experiments.

In Russia, Rubtzov (1932) placed five grasshopper species in cages and fed them on leaves of *Bromus inermis*. He found that an adult grasshopper eats 30% to 50% of its own weight in grass each day and that during its development an individual devours 20 times its weight in the adult stage. From this it was concluded that 607 pounds of grass per acre would be eaten by a population of 10 grasshoppers per square meter and that the total loss in eastern Siberia would amount to about 43,300 tons of hay per year.

Grasshopper feeding studies were also made by Langford (1930) who used a "photo-print" method to record surface areas of leaves before and after feeding by grasshoppers. His conclusion that "there is a wide daily variation in the amount of food eaten by individuals of the same brood hatched from the same batch of eggs and kept apparently under the same conditions" points out one of the difficulties encountered in attempting to interpret findings from laboratory studies of feeding by individuals of any single species in terms of a species complex occurring in nature.

Cages have been employed by various workers in attempts to measure losses caused by grasshoppers. Hinkle (1938) excluded grasshoppers from Colorado grassland areas by use of cages and then determined the amount of utilization by ocular comparisons of the amount of vegetation in the grasshopper-free cages with that on the outside of the cages. A similar use of cages had previously been made at 10 separate stations in the Crazy Mountains, Montana, by Morton (1936). Morton, however, made the comparisons by cutting vegetation both outside and inside the cages. He came to his conclusions on the amount of damage done by grasshoppers by taking the average of the measured damage at the 10 stations even though estimated grasshopper populations varied from 2 to 35 per square meter and the total damage to the vegetation varied from 9.3% to 97.3%. It is also noteworthy that in the data presented by Morton there appears to be little correlation between the number of grasshoppers per unit area and the amount of damage done: 15 grasshoppers per square meter destroyed 97.3%, 13 per square meter destroyed 20.3%, 25 per square meter destroyed 49.8%, and 35 per square meter destroyed 66.6%. In later studies in Montana and Wyoming, Morton (1939) used two cages at each station and compared the amount of vegetation produced in one cage in which grasshoppers had been placed with that in the second cage which remained grasshopper-free. In this study he recorded as high as 123% increase in vegetation, which was attributed solely to the effects of the cage upon the vegetation.

Pfadt (1949) also used cages to study grasshopper damage to rangeland vegetation. He used six 1-square-yard cages which remained grasshopper-free and 3 series of 6 cages with 15, 25, and 35 *Aulocara elliotti* (Thos.) per cage. At the end of the growing season the grass left in the cages was clipped, air-dried, and weighed. From these measurements Pfadt found a reduction of 406 pounds of grass per acre caused by 15 *A. elliotti* per square yard, a reduction of 489 pounds by 25 per square yard, and 792 pounds by 35 per square yard. In using *Melanoplus mexicanus*[3] instead of *A. elliotti* he found "the grass reduction was insignificant." However, the general conclusion was that 15 grasshoppers per square yard would reduce the forage an estimated 66%.

In recent studies by Nerney (1957) damage to grasses was evaluated from the percentage of blades, leaves and other plant parts eaten or cut off by grasshoppers or by a combination of both grassshoppers and cattle. From 200 to 300 grass blades were selected in each plot and "The damage to each grass blade was estimated by a close unit measurement scaled in tenths or hundredths in relation to blade lengths. Because dominant grasses have flat blades, length measurements can be closely estimated by a comparison of the width on damaged and undamaged blades or by the relative blade position on culms and stems. The average percentage of herbage eaten on damaged blades was multiplied by the percentage of blades fed on, giving the estimated percentage of herbage utilized for that point." These estimates of damage were related to the grasshopper populations present which were measured by use of a "pointer method" also described by Nerney (1957).

In 1950 and 1951 attempts were made in Montana (Anderson & Wright 1952) to measure grasshopper damage without the use of cages. Approximately 600 acres of livestock-free winter-grazing land were used in each of these studies. Grasshoppers remained on about half of each study area while the remaining portion was sprayed

[3] *M. mexicanus* is now considered to be *Melanoplus bilituratus* (Gurney & Brooks 1959). Throughout the paper, however, in quoting other workers the names used by them will be maintained.

Table 1.—Weights of forage from sprayed and unsprayed areas to measure grasshopper damage.[1] 1955.

Area and Vegetation	Sprayed			Unsprayed		
	Clipping 1	Clipping 2	Difference (2−1)	Clipping 1	Clipping 2	Difference (2−1)
Barber	(July 13)	(Sept. 7)		(July 14)	(Sept. 9)	
Bouteloua gracilis	141.6***	201.6	+ 61.0**	264.0***	314.4	+ 50.4**
Stipa comata	26.4	31.2	+ 4.8	153.6	52.8	−100.8
Agropyron smithii	172.8	12.0	−160.8	38.4	60.0	+ 21.6
Carex eleocharis	50.4	38.4	− 12.0	4.8	0.0	− 4.8
Barber	(July 14)	(Sept. 6)		(July 15)	(Sept. 8)	
Stipa comata	751.2***	696.0	− 55.2*	820.8***	717.6	−103.2**
Weeds	12.0	7.2	− 4.8	21.6	4.8	− 16.8
Loma	(July 27)	(Sept. 13)		(July 27)	(Sept. 12)	
Agropyron smithii	664.8***	669.6	+ 3.8*	835.2***	734.4	−100.8*
Stipa comata	96.4	67.2	− 29.2	139.2	64.8	− 74.4
Poa secunda	124.8	184.8	− 60.0	67.2	127.2	+ 60.0
Carex eleocharis	9.6	4.8	− 4.8	9.6	0.1	− 9.5
Weeds	36.0	38.4	+ 2.4	69.6	4.8	− 64.8
Harlowton	(July 21)	(Sept. 8)		(July 19)	(Sept. 8)	
Stipa comata	804.0***	640.8	−163.2**	384.0***	460.8	+ 76.8**
Bouteloua gracilis	0.5	4.8	+ 4.3	194.4	4.8	−189.6
Agropyron smithii	21.6	24.0	+ 2.4	55.2	21.6	− 33.6
Weeds	52.8	36.0	− 16.8	141.6	26.4	−115.2
Geraldine	(July 30)	(Sept. 15)		(July 28)	(Sept. 14)	
Bouteloua gracilis:						
Poa secunda +*Carex eleocharis*	1,173.6***	1,216.8	+ 42.2*	847.2***	1,003.2	+156.0**
Agropyron smithii	122.4	105.6	− 16.8	52.8	79.2	+ 26.4
Weeds	26.4	21.6	− 4.8	43.2	31.2	− 12.0

[1] Expressed in pounds of air-dried forage per acre.
* No significant difference.
** Significant difference.
*** Highly significant difference between sprayed and unsprayed clipping 1.

with an insecticide to eliminate them. Within both the sprayed and unsprayed portions sampling areas were selected which appeared to be comparable. Grasses and sedges were clipped at monthly intervals in the sampling areas and the production of each was thus measured and compared. An evaluation of the measured losses was made on the basis of observed feeding habits of the various grasshopper species comprising the population complex present. These earlier studies in Montana showed that an evaluation of the grass-damage potential of a particular grasshopper population on the basis of numbers alone is not valid and also indicated that a per cent damage figure by itself is meaningless unless total production of vegetation is considered.

The studies reported in this paper are patterned after those made in 1950 and 1951 in Montana on damage caused by grasshoppers. In some cases refinement of previous techniques for measuring and evaluating losses in vegetation and for measuring the grasshopper populations has been made.

STUDIES IN 1955.—Four separate areas were selected for study. In the Barber area, two distinct vegetation types were recognized and each treated separately. All of the areas were winter pastures, *i.e.*, grazing by livestock occurred during the late fall and winter months only and did not interfere with the measurements of seasonal damage caused by grasshoppers.

Approximately half of each study area was sprayed by aircraft to reduce the grasshopper populations to much less than one per square yard. Approximately 75 acres of the Harlowton area and 200 acres each of the Barber, Loma, and Geraldine areas were sprayed just prior to the first clipping of vegetation.

Areas which appeared to be alike from the standpoint of vegetation were selected in the sprayed and unsprayed portions of each major area for the measurement of yields. At the start of the study and again at the end of the study the vegetation in fifty 4-square-foot quadrats located at random within the areas to be compared was clipped at ground level, air-dried, and weighed. A summary of these data is found in table 1.

Grasshopper populations of each unsprayed area studied were measured by use of cages previously described by Anderson & Wright (1952). The cages were placed after nighttime temperatures became low, banked with dirt to prevent movement in or out, and left in place until morning when the grasshoppers thus trapped were removed and recorded as to species and number. On the Barber study area 20 grasshopper species were found with an average population of 14.8 grasshoppers per square yard during the period July 13 to August 13. The dominant species was *Cordillacris crenulata* (Bruner) which averaged 2.2 per square yard during the period. The highest population, averaging 38.4 per square yard, was found on the Loma study area. *Melanoplus bilituratus* (Walker), averaging 31.5 per square yard, was the dominant of the

19 species found on the area. From July 18 to September 10 the population on the Harlowton area averaged 16.6 per square yard, and 17 species were found with the dominants being *Melanoplus infantilis* Scudder (average 4.5/sq. yd.), *Ageneotettix deorum* (Scudder) (average 4.2/sq. yd.), and *M. bilituratus* (average 2.1/sq. yd.). The 18 species found on the Geraldine area averaged 13.9 per square yard during the month of August. The dominant species were *Amphitornus coloradus* (Thomas) (average 4.2/sq. yd.) and *M. bilituratus* (average 3.6/sq. yd.).

In evaluating the 1955 data given in table 1, a highly significant difference is found to exist between the amounts of the dominant vegetation of the first clipping in the sprayed and unsprayed areas, indicating that the samples taken from the sprayed portions were not comparable with those from the unsprayed portions. Secondly, a number of unexplainable discrepancies appear in the data, *e. g.*, western wheatgrass (*Agropyron smithii*) in the Barber-Blue grama area and in the Geraldine area, Sandberg bluegrass (*Poa secunda*) in the Loma area, and needle-and-thread (*Stipa comata*) in the Harlowton area decreased in weight between the first and second clippings in the sprayed areas and increased in weight in the unsprayed areas where grasshoppers were feeding. From this result one might conclude that the presence of grasshoppers was responsible for an increase in production of these grasses. These 1955 data point to the necessity of recognizing that the vegetation within an area is not randomly distributed. Therefore, random samples, at least in the numbers taken, did not give a reliable measure of the losses caused by grasshoppers.

STUDIES IN 1956.—One new area near Townsend and the area near Harlowton used in the 1955 studies were selected for study in 1956. Livestock-free winter pastures were again used for the studies and approximately half of each area was sprayed by aircraft to reduce grasshopper populations to much less than one per square yard. In the Townsend area 300 acres were sprayed on June 13, 1956. The Harlowton plot had originally been sprayed on July 14, 1955, and was still quite grasshopper-free at the start of the 1956 season. As the season progressed, however, the grasshopper population of this 75-acre plot approached one per square yard due to local movements from surrounding areas and it was deemed advisable to spray the area again on August 2, 1956.

In both of these study areas certain vegetation types were recognized for study within both the sprayed and unsprayed portions. These were recognized on the basis of the dominant vegetation within them and those vegetation types of the sprayed portion were selected as nearly comparable to those in the unsprayed portion as possible. At Townsend the three types studied were: *Bouteloua gracilis*, *Agropyron smithii*, and *Koeleria cristata*. The three types studied at Harlowton were: *Bouteloua gracilis*, *Agropyron smithii*, and *Stipa comata*. Measurements were made to determine the per cent of the total area comprised by each type and 50 pairs of 4-square-foot quadrats were selectively located in each so that the density and composition of the vegetation within each was as nearly like that of the next one to it as possible. Within each type of both the sprayed and unsprayed portions the vegetation in one quadrat of each of the 50 pairs was clipped at ground level at the beginning of the season, air-dried, and weighed and that in the remaining quadrat of each pair was treated similarly at the end of the season. An adequate amount of the subdominant vegetation of the types studied was not encountered in the quadrats clipped to be significant in an analysis of the amount of grasshopper damage. Consequently, only the dominant vegetation of each type is included in the summary in table 2.

The grasshopper populations of each unsprayed type of vegetation studied were again measured by the use of cages established at night. The method was modified somewhat from that previously described by placing a single cage at random no less than 18 times after nighttime temperatures had dropped to the point where the grasshoppers were relatively inactive. Immediately after each placement a gas lantern was put in the cage through a door in the side and all grasshoppers trapped were removed, killed, and saved for future study. Not only did the lantern provide adequate light but it also provided sufficient heat to activate the grasshoppers trapped. This change in method was of importance because it was previously noted that some of the species which burrow into debris or clumps of vegetation were not active enough to be located when light such as that from a flashlight was used. It was also found that by the immediate removal of the grasshoppers trapped it was not necessary to bank the edges of the cage with dirt to prevent movement in or out. Disturbance to the area was thus decreased. Other precautions against disturbance were taken such as not driving a vehicle over the areas of study, not trampling the vegetation by sitting on it, and not leaving any cages,

Table 2.—Mean weight of air-dried grass per quadrat clipped at Harlowton and Townsend areas.[1] 1956.

	Sprayed				Unsprayed					
Area and Vegetation	Clipping 1	Clipping 2	Difference (2−1) D, 1	t-Test for D, 1	Clipping 1	Clipping 2	Difference (2−1) D, 2	t-Test for D, 2	(D, 1−D, 2) D, 3	t-Test for D, 3
Harlowton	(June 25)	(Sept. 12)			(June 22)	(Sept. 12)				
B. gracilis	23.420	21.268	− 2.152	− 3.224**	19.000	12.794	− 6.206	− 5.762*	2.538	3.199**
A. smithii	23.706	19.534	− 4.172	− 7.736*	20.402	9.792	−10.610	−14.231*	6.495	7.039*
S. comata	19.680	15.294	− 4.386	−10.787*	17.898	6.049	−11.849	−34.636*	7.463	14.000*
Townsend	(June 13)	(Aug. 21)			(June 7)	(Aug. 23)				
B. gracilis	10.592	20.674	+10.082	10.110*	20.288	19.708	− 0.580	−0.309***	10.391	5.041*
A. smithii	12.872	23.666	+10.794	18.217*	14.288	9.558	− 4.730	−5.471*	15.524	8.394*
K. cristata	12.786	13.496	+ 0.710	1.272***	18.342	11.220	− 7.122	−9.506*	8.394	8.384*

[1] Grams per 4 square feet.
* $P < 0.001$.
** $0.001 < P < 0.01$.
*** Not significant.

Table 3.—Weights of forage from sprayed and unsprayed areas to measure grasshopper damage.[1] 1956.

Area and Vegetation	Average Number of Grasshoppers Per Sq. Yd.	Sprayed			Unsprayed			Corrected Loss	
		Clipping 1	Clipping 2	Difference (2−1)	Clipping 1	Clipping 2	Difference (2−1)	Weight	Per Cent
Harlowton									
B. gracilis	11.0	562.4	510.7	− 51.7**	456.3	307.3	−149.0*	107.0**	25.9
A. smithii	11.1	569.2	469.3	−100.1*	490.9	235.2	−254.8*	168.6*	41.8
S. comata	7.9	472.6	367.3	−105.3*	429.9	145.3	−284.6*	187.5*	56.2
Townsend									
B. gracilis	8.8	254.4	496.4	+242.0*	486.6	473.4	− 13.2***	476.9*	50.2
A. smithii	12.9	309.1	568.3	+259.2*	343.1	229.5	−113.6*	401.7*	62.1
K. cristata	11.4	307.0	324.1	+ 17.1***	440.4	269.4	−171.0*	197.4*	42.3

[1] Expressed in pounds of air-dried forage per acre.
* $P < 0.001$.
** $0.001 < P < 0.01$.
*** Not significant.

wooden stakes, or other debris in the area. Previous observations indicated that changes in grasshopper distribution and activities result from disturbances to an area, particularly that of trampling vegetation in localized spots.

The average grasshopper population found on the Harlowton *Bouteloua gracilis* type was 11.0 per square yard from July 10 to September 6. *Ageneotettix deorum* (average 4.5/sq. yd.) and *Phlibostroma quadrimaculatum* (Thomas) (average 2.5/sq. yd.) were the dominants of the 17 species found. *A. deorum* (average 4.8/sq. yd.) and *Aulocara elliotti* (average 2.1/sq. yd.) were dominants of the 14 species found on the Harlowton *Agropyron smithii* type. The average population on this type was 11.1 per square yard during the study period. The Harlowton *Stipa comata* type had fewer grasshoppers with an average of 7.9 per square yard. *A. deorum* (average 2.8/sq. yd.) and *A. elliotti* (average 1.7/sq. yd.) were the dominants of the 12 species found.

Seventeen species were found on the Townsend *Bouteloua gracilis* type with an average population of 8.8 per square yard from June 12 to August 21. *A. elliotti* (average 4.0/sq. yd.) was dominant and was followed by *Melanoplus occidentalis* (Thomas) and *M. bilituratus* each averaging 1.5 per square yard. The average population on the Townsend *Agropyron smithii* type was 12.9 per square yard. *A. elliotti* (average 4.7/sq. yd.) was again the dominant of the 14 species found on this type. It was followed in importance by *M. occidentalis* (average 3.6/sq. yd.) and *M. bilituratus* (average 2.0/sq. yd.). The Townsend *Koeleria cristata* type supported an average population of 11.4 per square yard. The 13 species found on this area were dominated by *A. elliotti* (average 4.8/sq. yd.). Other species of importance on this type were *M. bilituratus* (average 1.9/sq. yd.), *M. occidentalis* (average 1.7/sq. yd.), and *M. infantilis* (average 1.1/sq. yd.).

A summary of the measurements of the 1956 damage is found in table 3. The "corrected loss" assumes that, since the sprayed and unsprayed areas were comparable, what happened in the way of gain or loss of vegetation in the sprayed portion during the season would also have taken place in the unsprayed portion if grasshoppers had not been present. The "corrected loss" was thus calculated as follows:

$$\text{Corrected loss} = \left(\frac{\text{Sprayed clipping 2}}{\text{Sprayed clipping 1}} \times \text{Unsprayed clipping 1}\right) - \text{Unsprayed clipping 2}$$

For example, the corrected loss for the Harlowton *Bouteloua gracilis* type is:

$$\left(\frac{510.7}{562.4} \times 456.3\right) - 307.3 = 107 \text{ lb. per acre}$$

As previously mentioned, the unsprayed areas were surveyed to determine the approximate percentage of the total area occupied by such vegetational type. At Harlowton *B. gracilis* amounted to approximately 12%, *A. smithii* 11%, and *S. comata* 77% of the total study area. At Townsend *B. gracilis* occupied approximately 7%, *A. smithii* 27%, and *K. cristata* 66% of the total study area. These figures along with the measurements of damage indicate that the damage over a large area is not uniform but may vary from one localized portion to the next. One may observe similar variations in the amount of damage within portions of a single vegetation type.

From observations during these and previous studies on the feeding habits of grasshoppers (Anderson & Wright 1952) it is possible to assign most of the responsibility for the damage done in each type of vegetation to certain of the grasshopper species present. Five of the 17 species present on the Harlowton *Bouteloua gracilis* type were responsible for most of the damage; the greatest amount probably was done by *Aulocara elliotti* (average 1/sq. yd.) and *Melanoplus infantilis* (average 0.8/sq. yd.). In the order of their importance, these two species were followed by *Phlibostroma quadrimaculatum* (average 2.5/sq. yd.), *Amphitornus coloradus* (average 0.5/sq. yd.), and *Ageneotettix deorum* (average 4.5/sq. yd.). The listed order of importance of these species is not the same as their order of abundance. Although *A. elliotti* is commonly more numerous in wheatgrass (*Agropyron* spp.) areas it is a voracious grass-feeder which not only devours but also cuts off numerous grass leaves which fall to the ground. *M. infantilis* appears to prefer areas of *B. gracilis* and feeds heavily on this grass species. *P. quadrimaculatum* has a definite preference for *B. gracilis* but appears to spend little time in feeding as an adult. *A. coloradus* feeds

heavily on *B. gracilis*. The most numerous species present was *A. deorum*. It is a grass-feeding species with no particular host preference shown and it includes large amounts of dry or drying plant debris in its diet.

Most of the losses in the Harlowton *Agropyron smithii* type may be attributed to feeding by *Aulocara elliotti* (average 2.1/sq. yd.). *Metator pardalinus* (Sauss.) (average 0.6/sq. yd.), *M. infantilis* (average 0.5/sq. yd.), and *Ageneotettix deorum* (average 4.8/sq. yd.) were also responsible for some of the damage. *A. elliotti* (average 1.7/sq. yd.) was also responsible for much of the loss in the Harlowton *Stipa comata* type. Other species contributing to the loss were *A. deorum* (average 2.8/sq. yd.), *M. infantilis* (average 0.8/sq. yd.), *Melanoplus occidentalis* (average 0.4/sq. yd.), and *A. coloradus* (average 0.3/sq. yd.).

Nearly all of the losses from grasshoppers on the Townsend *Bouteloua gracilis* type were caused by *Aulocara elliotti* (average 4.0/sq. yd.) and *Melanoplus occidentalis* (average 1.5/sq. yd.). Although *Melanoplus bilituratus* (average 1.5/sq. yd.) is known to feed on various grasses, it shows a definite preference for forbs as an adult and probably contributed no more to the loss of *B. gracilis* than did *Trachyrhachys kiowa* (Thomas) (average 0.3/sq. yd.) and *M. infantilis* (average 0.3/sq. yd.).

A. elliotti (average 4.7/sq. yd.) and *M. occidentalis* (average 3.6/sq. yd.) were also responsible for most of the losses on the Townsend *Agropyron smithii* type. Although comparatively numerous, *M. bilituratus* (average 2.0/sq. yd.) was probably responsible for only a minor part of the reduction in *Agropyron smithii* during the season.

Losses in the Townsend *Koeleria cristata* type were also caused by *A. elliotti* (average 4.8/sq. yd.) and to a lesser extent *M. occidentalis* (average 1.7/sq. yd.). *M. infantilis* (average 1.1/sq. yd.) also aided in the destruction. *M. bilituratus* (average 1.9/sq. yd.) probably accounted for little more than the early season losses inflicted by *Aeropedellus clavatus* (Thomas) (average 0.3/sq. yd.), an early hatching and early maturing species.

STUDIES IN 1957.—These studies were carried out on the Fishtail Ranch of the Kiewit Cattle Company on a high east-west lateral moraine approximately 10 miles northwest of the town of Big Timber at the base of the Crazy Mountains. The area was not grazed during the spring and summer and few cattle used it during the fall months. A ravine divides the moraine approximately in half longitudinally. The north half (150 acres) was sprayed by aircraft on July 2 with 1 ounce of dieldrin in 1 gallon of oil per acre. Within 24 hours the grasshopper population on the sprayed area was much less than one per square yard and remained in very low numbers throughout the study period.

Sites which appeared to be comparable from the standpoint of vegetation were selected in both the sprayed and unsprayed portions of the area for measurements of production. Lichens were collected and the remaining vegetation was clipped at ground level in each of 25 four-foot quadrats, then air-dried, and weighed. These quadrats were located at 10-foot intervals in a straight line in both the sprayed and unsprayed study sites. Clippings on succeeding dates were located 4 feet from the previous clipping along a line parallel to the original line; so that in this study the clippings conformed to a grid pattern. Thus, each quadrat was not selected at random although the grid which determined the quadrat site was selected at random within the two portions of the study site which appeared to be comparable. A summary of the clipping data is presented in tables 4 and 5.

The grasshopper population on the unsprayed portion of the study area was measured by use of cages as previously described for the 1956 studies. Twenty cages were placed on the unsprayed area nearly every other night from July 2 to August 17. The population, comprised of 20 species, averaged 14.5 grasshoppers per square yard during this period. The dominant species was *Aulocara elliotti* (average 3.6/sq. yd.), *Melanoplus packardii* Scudder (average 2.2/sq. yd.), and *Ageneotettix deorum* (average 2.0/sq. yd.).

The 1957 studies were terminated by a severe hail storm accompanied by high winds the night of August 17. The vegetation on both sprayed and unsprayed portions of the study area was severely damaged and the grasshopper population of the unsprayed area was greatly reduced. Within a week after the storm it appeared that much of the hail-damaged vegetation of the grasshopper-free sprayed portion had recovered whereas vegetation of the unsprayed portion previously damaged by grasshopper feeding did not seem to make a similar recovery and considerable bare ground along with areas covered by windrows of plant debris and standing skeletons of plants characterized the area.

The two portions of the study area were considerably different at the end of the season with obvious grasshopper feeding having occurred on the unsprayed portion. An analysis of the clipping data, however, does not show that an accurate measure of the amount of damage was made. Data presented in table 4 show that significant differences existed between all but four of the common grass species found on the two areas at the time of the first clipping; consequently, only the four species were compared. At the end of the season a significant difference in weight between the sprayed and unsprayed *Agropyron smithii* was measured. Although the gain in weight of *A. smithii* on the sprayed portion and its loss in weight on the unsprayed portion were not significant changes during the season, the difference of 101.6 pounds per acre between the two areas at the end of the season was highly significant. The corrected loss, calculated as described above amounted to 89 pounds (air-dry) per acre and may be attributed mainly to *Aulocara elliotti*. The following species also contributed somewhat to the losses: *Melanoplus infantilis* (average 0.7/sq. yd.), *Metator pardalinus* (average 0.6/sq. yd.), *Bruneria brunnea* (Thomas) (average 0.1 /sq. yd.), *Amphitornus coloradus*, and *Ageneotettix deorum*.

A number of the grasshopper species present on the area fed on *Koeleria cristata*. In the approximate order of their importance, those species were: *Aeropedellus clavatus* (average 0.4/sq. yd.), *Arphia pseudonietana* (Thom.) (average <0.1/sq. yd.), *Aulocara elliotti*, *Ageneotettix deorum*, *Melanoplus bilituratus* (average 0.5/sq. yd.), *Amphitornus coloradus*, and *Metator pardalinus*. Of these, only the early hatching *A. clavatus* showed any preference for *K. cristata*. In spite of the presence of these species, both the sprayed and unsprayed areas of *K. cristata* had increases in yield during the season. The difference be-

Table 4.—Weights of grasses and sedges from sprayed and unsprayed areas to measure grasshopper damage.[1] 1957.

Plant Species	Clipping 1 July 3	Clipping 2 July 16	Clipping 3 July 29	Clipping 4 Aug. 13	Difference 1–2	Difference 1–3	Difference 1–4
Agropyron spicatum							
Sprayed	329.5						
Unsprayed	149.7						
Difference	+179.8***						
Agropyron smithii							
Sprayed	175.0	181.3	234.9	235.4	− 6.3*	−59.9*	−60.4*
Unsprayed	159.2	125.0	126.1	133.8	+34.2	+33.1*	+15.4*
Difference	+ 15.8*	+ 56.3*	+108.8***	+101.6***			
Koeleria cristata							
Sprayed	149.1	189.3	165.3	179.3	−40.2*	−16.2*	−30.2*
Unsprayed	124.6	173.2	182.2	144.0	−48.6*	−57.6**	−19.4*
Difference	+ 24.5	+ 16.1*	− 16.9*	+ 35.3*			
Stipa viridula							
Sprayed	20.6	4.7	8.4	11.6	+15.9*	+12.2*	+ 9.0*
Unsprayed	56.0	33.3	27.0	11.5	+22.7*	+29.0*	+44.5*
Difference	− 35.4*	− 28.6***	18.6**	+ 0.1*			
Stipa comata							
Sprayed	23.1						
Unsprayed	70.3						
Difference	− 47.2**						
Poa cusickii							
Sprayed	60.1						
Unsprayed	7.2						
Difference	+ 52.9***						
Poa secunda							
Sprayed	101.7						
Unsprayed	31.7						
Difference	+ 70.0***						
Total grasses and sedges							
Sprayed	866.6						
Unsprayed	615.8						
Difference	+250.8***						

[1] Expressed in pounds of air-dried forage per acre.
* No significant difference.
** Difference significant at 5% level.
*** Difference significant at 1% level.

tween the two portions of the area, however, was not significant.

A loss in the yield of *Stipa viridula* was recorded for both the grasshopper-free sprayed area and the unsprayed area. The difference between the two areas was not significant at the end of the season, although significant differences were measured by Clipping 2 and Clipping 3 during midseason. The loss on the unsprayed area could be attributed to the presence of grass-feeding grasshopper species but does not explain the decrease in *S. viridula* from the sprayed area which is even more puzzling because it is a rather late-maturing species which continues vigorous growth until late summer.

Losses in weight, although not significant, were also recorded for *Poa secunda* growing on the treated and untreated areas. *P. secunda* normally grows quite rapidly in the spring, matures early, and then starts to dry up. The pattern of growth indicated by the clippings on the sprayed area is what one would expect, with growth continuing and increases in weight until Clipping 2 followed by the drying-up of the grass resulting in a loss in weight. The continued loss of weight on the unsprayed area is also to be expected since a number of grass-feeding species, particularly *Aulocara elliotti* and *Melanoplus infantilis*, ate this grass. The combination of grasshopper feeding and normal maturing of the grass thus results in a loss in weight during the season. When the total weights of grasses and sedges growing on the study area were considered, a highly significant difference between the two areas was recorded at the beginning of the season and, consequently, could not be compared throughout the season.

Nine of the 10 most common forbs of the area were found in comparable amounts on the two areas at the beginning of the season (table 5). Significant differences in the amounts of only four of these forbs were recorded at the end of the study. It was found that *Phlox hoodii* decreased in weight on both the sprayed and unsprayed areas but was not found to have changed significantly. Very little feeding by grasshoppers upon this plant has been observed and it is felt that very little of the loss on the unsprayed area could be attributed to grasshoppers.

Sampling errors caused by the nonrandom distribution of *P. hoodii* probably accounts for changes on the unsprayed area recorded by the second and third clippings.

Vicia sparsifolia is one of the preferred food plants of *Melanoplus packardii*, *M. bilituratus*, *M. bivittatus* (Say) (average 0.2/sq. yd.), and *M. confusus* Scudder (average <0.1/sq. yd.), all of which were present on the unsprayed portion of the study area. However, the loss in weight of *V. sparsifolia* was not significant on this portion but was significant where there was no grasshopper feeding. The

Table 5.—Weights of forbs and lichens from sprayed and unsprayed areas to measure grasshopper damage.[1] 1957.

Plant Species	Clipping 1 July 3	2 July 16	3 July 29	4 Aug. 13	Difference 1-2	1-3	1-4
Phlox hoodii							
Sprayed	357.1	431.2	384.1	325.6	− 74.1*	− 27.0*	+ 31.5*
Unsprayed	394.6	291.1	514.2	300.9	+103.5*	−119.6*	+ 93.7*
Difference	− 37.5*	+140.1**	−130.1*	+ 24.7*			
Vicia sparsifolia							
Sprayed	94.4	89.4	69.4	32.8	+ 5.0*	+ 25.0*	+ 61.6**
Unsprayed	92.3	87.7	73.3	54.7	+ 4.6*	+ 19.0*	+ 37.6*
Difference	+ 2.1*	+ 1.7*	− 3.9*	− 21.9**			
Astragalus drummondii							
Sprayed	257.4	236.6	299.9	166.4	+ 20.8*	− 42.5*	+ 91.0*
Unsprayed	106.2	10.7	34.8	6.9	+ 95.5**	+ 71.4*	+ 99.3**
Difference	+151.2*	+225.9***	+265.1***	+159.5**			
Arenaria congesta							
Sprayed	90.9	176.2	151.0	94.7	− 85.3*	− 60.1*	− 3.8*
Unsprayed	70.2	125.3	47.9	61.3	− 55.1*	+ 22.3*	+ 8.9*
Difference	+ 20.7*	+ 50.9*	+103.1**	+ 33.4*			
Sphaeralcea coccinea							
Sprayed	9.6	13.6	21.6	19.4	− 4.0*	− 12.0**	− 9.8**
Unsprayed	11.5	5.6	7.5	9.1	+ 5.9*	+ 4.0*	+ 2.4*
Difference	− 1.9*	+ 8.0***	+ 14.1***	+ 10.3**			
Allium textile							
Sprayed	5.0	4.9	2.8	3.7	+ 0.1*	+ 2.2*	+ 1.3*
Unsprayed	8.2	2.3	1.8	1.4	+ 5.9***	+ 6.4***	+ 6.8***
Difference	− 3.2*	+ 2.6**	+ 1.0*	+ 2.3*			
Viola nuttallii							
Sprayed	4.1	4.0	1.9	1.5	+ 0.1*	+ 2.2**	+ 2.6***
Unsprayed	4.4	4.2	1.4	0.4	+ 0.2*	+ 3.0*	+ 4.0**
Difference	− 0.3*	− 0.2*	+ 0.5*	+ 1.1*			
Lomatium foeniculaceum							
Sprayed	11.7	3.7	4.2	4.0	+ 8.0*	+ 7.5*	+ 7.7*
Unsprayed	17.0	3.0	0.7	1.4	+ 14.0***	+ 16.3***	+ 15.6***
Difference	− 5.3*	+ 0.7*	+ 3.5***	+ 2.6**			
Hymenoxys acaulis							
Sprayed	16.1	8.0	14.3	34.8	+ 8.1*	+ 1.8*	− 18.7*
Unsprayed	40.0	44.1	21.0	7.3	− 4.1*	+ 19.0*	+ 32.7**
Difference	− 23.9*	− 36.1**	− 6.7*	+ 27.5*			
Artemisia frigida							
Sprayed	0.6						
Unsprayed	69.9						
Difference	− 69.3***						
Total forbs							
Sprayed	876.8	1,093.6	1,055.3	750.9	−216.8*	−178.5*	+125.9*
Unsprayed	857.6	664.2	800.9	527.2	+193.4**	+ 56.7*	+330.4**
Difference	+ 19.2*	+429.4***	+254.4*	+223.7**			
Lichens							
Sprayed	31.1	30.4	38.5	27.7	+ 0.7*	− 7.4*	+ 3.4*
Unsprayed	37.9	57.3	71.7	77.4	− 19.4*	− 33.8**	− 39.5***
Difference	− 6.8*	−26.9***	− 33.2**	− 49.7***			

[1] Expressed in pounds of air-dried forage per acre.
* No significant difference.
** Difference significant at 5% level.
*** Difference significant at 1% level.

Table 6.—Yields of western wheatgrass at OW Ranch, 1950–59.[1]

	Year									
	1950[2]	1951	1952	1953	1954	1955	1956	1957	1958	1959
					Sprayed area					
First clipping (June 10–15)	442	484	439	789	300	199	581		422	478
Second clipping (Sept. 5–10)	509	509	452	645	187	706	540	468	797	386
Difference	+ 67	+ 25	+ 13	−144	−113	+507	− 41		+375	− 92
					Unsprayed area					
First clipping (June 10–15)	327	337	273	536	163	204	566		343	408
Second clipping (Sept. 5–10)	124	405	336	417	113	384	449	346	706	422
Difference	−203	+ 68	+ 61	−119	− 50	+180	−117		+363	+ 14

[1] Expressed in pounds of air-dried forage per acre.
[2] Grasshoppers present.

difference in amounts present on the two areas at the end of the season was also significant. Normally, even with no grasshoppers present, one might expect losses of *Vicia* during a season because of the drying-up of the plants and consequent breakage and loss of plant parts. The difference recorded between the two areas is not easily explained. It is a possibility that the feeding by grasshoppers on the unsprayed area stimulated growth of *V. sparsifolia* by removal of vegetation which on the sprayed area competed for light. Thus, the feeding on *Vicia* in the unsprayed area may have been masked to some extent by increased growth of this species.

Astragalus drummondii was eaten extensively by some species of *Melanoplus* mentioned above, particularly *M. packardii* and *M. bilituratus*. These two species were responsible for most of the corrected loss of 61.8 pounds per acre.

The growth and seasonal history of *Arenaria congesta* on the study area was very similar to that of *Phlox hoodii*. No significant difference in its weight from the sprayed and unsprayed areas was recorded at the end of the study period.

A significant increase in the weight of *Sphaeralcea coccinea* was measured on the sprayed area. At the same time a decrease was recorded for the unsprayed area. This decrease (corrected loss of 14.1 pounds per acre) can be attributed to nearly all of the forb-feeding grasshopper species present. No single species is known to prefer *Sphaeralcea* or to concentrate periods of feeding upon it. Those species known to have contributed to the loss of *S. coccinea* are: *Cratypedes neglectus* (Thomas), (average 0.2/sq. yd.), *Hadrotettex trifasciatus* (Say) (average 0.2/sq. yd.), *Melanoplus bivittatus*, *M. bilituratus*, *M. confusus*, *M. packardii*, *Spharagemon equale* (Say) (average <0.1/sq. yd.) and *Trimerotropis campestris* McNeill (average <0.1/sq. yd.).

Highly significant losses of *Allium textile* occurred on the unsprayed area whereas the losses on the sprayed area were not significant. However, no significant difference between the two areas was found when they were compared at the end of the study. This forb matures and starts drying by midsummer which would account for the losses in both areas. Feeding by grasshoppers also may have accounted for some of the loss on the unsprayed area although only occasional slight feeding on this plant was observed.

Viola nuttallii also matures early and starts drying by midsummer, thus accounting for losses in weight by this species in both areas. It is known that *Melanoplus bilituratus* in particular feeds on this plant and accounted for some of its loss on the unsprayed area. However, no significant difference between the two areas was measured.

Lomatium foeniculaceum matures early, dries, and becomes quite inconspicuous in an area early in the summer. Although losses of this forb were measured in both areas, those on the unsprayed were found highly significant. A significant difference also was measured at the end of the study between the two areas, amounting to a corrected loss of 4.4 pounds per acre. Most of the feeding on *Lomatium* by grasshoppers and the consequent loss in weight occurred after it had dried. The feeding was not only by those species which are normally forb-feeders but also included a number of the grass-feeding species which feed on dry plant debris on the ground and which climbed upon the dried *Lomatium* plants to feed.

No significant difference was measured in the amounts of *Hymenoxys acaulis* found on the sprayed and unsprayed areas at the end of the study. It is felt that the recorded changes which occurred on the two areas may be attributed primarily to sampling error caused by the distribution of this plant and that the data do not warrant further consideration.

Data given in table 5 indicate that when the total weight of all forbs encountered on the plots was considered, no significant change occurred on the sprayed area. A significant loss, however, occurred on the unsprayed area which may be attributed to the feeding by grasshoppers which amounted to a corrected loss of 210.3 pounds per acre.

Foliose lichens were quite common over the area at the beginning of the study. No significant change took place on the sprayed area during the study. However, where grasshoppers continued to feed there was an obvious significant increase in the amount of lichens as the season progressed which amounted to a corrected gain of 43.7 pounds per acre. Only occasional grasshopper feeding on lichens was observed and it would appear that the

removal of other vegetation by grasshoppers had made this portion of the area favorable for the growth of lichens.

DISCUSSION.—The studies reported in this paper have brought out a number of the problems encountered in attempting to measure seasonal losses of rangeland vegetation resulting from feeding by grasshoppers. Possibly the greatest of these problems involves the fact that neither the grasshoppers nor the plants are distributed at random within any area. The efficacy of applying random sampling methods and analyses to studies of this kind is therefore highly questionable.

In a selected area, it is felt that on any particular date a fairly accurate estimate of the grasshopper population may be obtained by the use of an adequate number of cages established at night. However, changes in the population occur quite rapidly during the season and, consequently, other than as a comparative figure, an average population density figure is rather meaningless in terms of vegetation loss.

The measurement of losses in yield of vegetation is also complicated by the uneven pattern of feeding displayed by grasshoppers which goes beyond the uneven distribution of both the grasshoppers and the vegetation and of the food-plant preferences of the individual species. It has been observed on numerous occasions that grasshoppers may congregate on certain individual plants or on a group of plants to feed and ignore neighboring plants belonging to the same species. The result is a mosaic pattern of severely damaged and relatively untouched plants within an area which itself is a mosaic of plant and grasshopper distribution.

Another problem involved in evaluating data on measurements of production from what appears to be comparable areas to determine losses of vegetation is indicated by data presented in table 6. These measurements of production were made on the sprayed and unsprayed, livestock-free, western wheatgrass (*Agropyron smithii*) areas established for the 1950 OW Ranch studies (Anderson & Wright 1952). With the exception of the first year, 1950, when the grasshoppers were present on the unsprayed area, populations remained much below one per square yard on both areas. In addition to the obvious differences in production by the two areas it is noteworthy that considerable reduction in yield of vegetation may take place during a season without any grazing by either livestock or grasshoppers.

As stated previously, the differential feeding by grasshoppers caused by the differences in food-plant preference makes the number of grasshoppers per unit area a poor basis for predicting losses in vegetation. In addition, there are some indications that whether a population is vigorous and increasing or lethargic and decreasing may have an effect upon the amount of vegetation destroyed. The impression is that when a population is building up in numbers there is less strife between individuals and more time is spent in feeding. Conversely, a population which has reached peak numbers or is decreasing seems to be more excitable and less time is spent in feeding. Considerable research must be carried out to determine whether these impressions are correct and to determine the importance of the physiological status of grasshoppers in evaluating their behavior including the amount of damage to vegetation for which they are responsible.

Possibly the best measure of the loss of vegetation was accomplished in the 1956 studies by use of selected paired quadrats. Even in such a study it was not possible to get an approximation of losses to more than the three dominant grass species of the area. Although the remaining vegetation may have been of little importance from the standpoint of total production of the area, its presence or absence as a factor influencing grasshopper damage must be known before data, such as that obtained in 1956, may be used to predict damage under similar situations.

Finally, to use the findings taken under one set of conditions in one area to predict what may take place in another one under different, though similar, conditions is at best not without error. Likewise, it is fallacious to calculate the amount of damage over a large area based on the findings in a small area. At the present time, no general predictions of losses to vegetation can be made. Each area under question must be considered and evaluated separately. Admittedly, even then any evaluation and prediction can be little more than an intelligent guess based on the species of grasshoppers present, their feeding habits and abundance, and on a knowledge of the vegetation present and its relative importance.

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Laboratory and Field Investigations with Phorate-Treated Cotton Seeds[1]

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Abstract

The absorption of P^{32}-labeled phorate by cotton seeds was investigated under laboratory, greenhouse, and field conditions. Dehulled seeds absorbed much more phorate than intact seeds; however, this increased amount of phorate in the dehulled seed did not reduce germination or radicle growth. Absorption of inorganic P^{32} was not appreciably inhibited by the seed coat. Water absorption and respiration of germinating cotton seeds was not markedly different between untreated and treated seeds. Cotton plants grown in sand and soil from phorate-treated seeds absorbed 2% to 6% of the applied dosage, chiefly during the first 7 days after planting.

Treatment of cotton seeds with phorate for control of insects attacking seedling cotton plants has been used for several years. Promising early season control of thrips (*Frankliniella* sp.), the cotton aphid (*Aphis gossypii* Glov.), cotton fleahopper (*Psallus seriatus* (Reut.)), southern garden leafhopper (*Empoasca solana* DeL.), and several other pests has been obtained with phorate seed treatment (Parencia *et al.* 1957, 1957a, 1958, Reynolds *et al.* 1957). The toxicant usually is applied as a 50% carbon dust at a dosage of 1 pound of phorate per 25 pounds of seeds (equivalent to 1 pound per acre).

The major disadvantage of planting phorate-treated cotton seeds is that poor stands of cotton are obtained when the germination and emergence period is cool and damp (Hacskaylo & Ranney 1957, Parencia *et al.* 1957). The present work was undertaken to evaluate critically the absorption and translocation of phorate following seed treatment under laboratory, greenhouse, and field conditions. An explanation of the poor emergence of phorate-treated cotton seeds under cool, damp conditions also was sought.

Materials and Methods.—Except where noted P^{32}-labeled phorate[4] with an original specific activity of approximately 2 millicuries per gram was used. Acid-delinted Deltapine-15 cotton seeds were treated with 4 mg. of P^{32}-labeled phorate per seed, which approximated the field dosage of 1 pound of phorate per 25 pounds of seeds. The phorate was applied as a 50% carbon dust with 3% methyl cellulose as a sticker. Except where noted the plants were harvested, dried at 60° to 70° C. for 24 hours, and ground in a cutting mill to pass through a 40-mesh screen. Aliquots (100 to 200 mg.) of the dried, ground plant material were weighed into stainless steel planchets for radioassay. Radioassay measurements were made with a Geiger-Mueller end-window tube and a conventional scaling unit, corrected for decay, and calculated as phorate-equivalents.

Laboratory Experiments.—Intact and manually dehulled seeds, both treated and untreated, were held in petri dishes at 65°, 75°, 85° and 95° F. for germination. Two pieces of Whatman No. 1 filter paper were placed in the bottom of each dish and 5 ml. of distilled water was added to provide moisture. Ten seeds were placed in each dish. The dehulled seeds were used in an attempt to increase phorate absorption sufficiently to reduce germination. Inorganic P^{32} (as $H_3P^{32}O_4$) was applied to cotton seeds by the same method used with the phorate. These were considered as untreated controls. All seeds were held in the dark. Groups of seeds were harvested at intervals and the seed coats removed. The embryos were rinsed in water and acetone, air-dried, and radioassayed. Only seeds that appeared to be germinating were radioassayed.

Intact seeds treated with nonradioactive technical phorate and untreated seeds were held at 65° and 85° F., as described above. These were weighed individually at 0, 24, 48, and 72 hours. The changes in weight of the seeds were used as an estimate of the water absorbed. Fifteen seeds were used for each treatment.

Respiration of germinating treated and untreated dehulled cotton seeds was estimated by oxygen consumption. Nonradioactive technical phorate and standard manometric techniques were employed. Measurements were made after the seeds had germinated for 48 and 72 hours at 85° F. A reaction temperature of 33° C. (91° F.) was used. Four germinating seeds were used for each measurement.

Greenhouse Experiments.—A group of cotton seeds treated with P^{32}-labeled phorate was planted in sand in the greenhouse (80° to 95° F.) in 1-pint cartons (10 per carton) and in 2-gallon crocks (5 per crock). Germinating seeds and emerging seedlings in the cartons were harvested from 1 to 7 days and the seedlings in the crocks from 7 to 35 days after planting. Nutrient solution was added to the containers as required. The seed coats and embryos were rinsed with water and acetone to remove any external phorate and air-dried prior to radioassay. The seedlings and plants were handled as described above.

Field Experiment.—One hundred P^{32}-labeled phorate-treated cotton seeds were planted in a 10-foot row in Lufkin fine sandy loam soil. Five randomly selected plants were harvested 1, 2, 3, 4, and 6 weeks after planting. As much of the root system as could be pulled up was included with each. These plants were handled individually.

Results.—*Laboratory Experiments.*—The most striking difference noted in the results of the petri-dish tests was the relatively large amount of phorate absorbed by the dehulled seeds compared with the intact seeds (table 1). This higher phorate content did not reduce either germination or radicle growth. Germination of the intact seeds was reduced by the phorate treatments. The percentage of germination of intact seeds at 5 days appeared to be lower than at 2 days, in some cases, because of the difficulty in determining when germination actually took place. The amount of phorate absorbed by the intact

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[4] Synthesized by Volk Radiochemical Co., 5412 N. Clark St., Chicago, Ill.

Table 1.—Germination, radicle length, and radioactivity absorbed by Deltapine-15 cotton seeds treated with P^{32}-labeled phorate or inorganic P^{32}.[a]

Condition of Seed and Radioactive Material Applied	Temperature (° F.)	Per Cent of Germination After—		Radicle Length (mm.) at—		Per Cent of Phorate or Inorganic P^{32} Absorbed After—		
		2 Days	5 Days	2 Days	5 Days	1 Day	2 Days	5 Days
Intact, phorate	65	70	30	1	26	0.3	0.4	2.4
	75	50	40	6	32	.3	.8	3.4
	85	60	80	4	14	.6	.7	2.5
	95	60	40	2	2	.5	1.0	1.9
Dehulled, phorate	65	80	90	2	21	8.0	13.9	13.9
	75	90	100	7	33	11.4	11.9	13.4
	85	100	—	14	—	10.4	17.5	—
	95	100	—	13	—	13.9	19.2	—
Intact, inorganic P^{32}	65	—	80	—	14	13	—	26
	75	—	80	—	29	14	—	44
	85	90	—	23	—	17	39	—
	95	80	—	22	—	19	33	—
Dehulled, inorganic P^{32}	65	—	100	—	24	20	—	31
	75	—	100	—	40	26	—	42
	85	90	—	30	—	27	38	—
	95	100	—	25	—	26	37	—

[a] Based on an average of 5 to 15 germinating seeds. The seeds were held in petri dishes in the dark.

seeds in this experiment was intermediate between that absorbed by intact seeds planted in soil and sand. In all petri-dish tests a greater percentage of dehulled seeds germinated than intact seeds. No data are shown for dehulled seeds at 85° and 95° F. at 5 days because they molded. Inorganic P^{32} was absorbed more rapidly and in a higher percentage than phorate. The seed coat did not reduce the percentage of inorganic P^{32} absorbed as much as it did phorate. In general, phorate and P^{32} absorption increased with a higher germinating temperature.

The changes in weight of intact phorate-treated and untreated cotton seeds held at 65° and 85° F. are shown in table 2. These changes were caused mostly by water absorption and are considered as such in the following discussion. Untreated seeds held at 65° F. absorbed approximately 10% more water during the first 24-hour period than those treated with phorate. This difference was significant at the 1% level. No differences were noted during the second and third 24-hour periods. The treated and untreated seeds held at 85° F. absorbed the same amount of water during the first 24-hour germinating period. However, during the 24- to 48-hour interval the untreated seeds absorbed more water. This difference was significant at the 2% level. It is interesting to note the similarity in amounts of water absorbed by the untreated seeds held at 65° and 85° F.

Table 2.—Effect of two temperatures on weight changes of intact phorate-treated and untreated cotton seeds.[a]

Germinating Temperature (° F.)	Average Milligrams of Weight Change per Seed with Standard Deviations from—			Number of Seeds Germinated at 72 Hours
	0–24 Hours	24–48 Hours	48–72 Hours	
	Untreated seeds			
65	102±12	− 4± 4	2± 4	2
85	98± 9	27±40	5±12	12
	Treated seeds			
65	90±12	2± 5	−1± 2	0
85	101±15	− 2±11	2±12	5

[a] Each figure is an average of 15 seeds.

The respiration experiments with dehulled phorate-treated and untreated cotton seeds showed no differences. After a 48-hour germination period the treated and untreated seeds consumed 1.2 and 1.3 microliters of oxygen per hour per gram of dry weight, respectively; after a 72-hour germination period the rates were 3.4 and 3.5 microliters, respectively.

Greenhouse Experiments.—The results of the experiment in which phorate-treated cotton seeds were planted in sand are summarized in table 3. These plants absorbed less phorate than those grown from phorate-treated seeds planted in soil. It was apparent from this experiment that most of the phorate was taken up and the most rapid absorption occurred during the 3 days immediately after planting. The phorate-equivalent in the cotyledons remained essentially constant from 1 to 5 weeks after planting. A small increase was found in the true leaves between 3 and 5 weeks. The amounts of chloroform-extractable radioactivity (phorate and toxic metabolites) in the cotyledons at 3, 4, and 5 weeks after planting were 30, 28, and 24 μg., respectively; on a fresh-weight basis these were equivalent to 67, 96, and 54 p.p.m., respectively.

Intact untreated, intact phorate-treated, dehulled untreated, and dehulled phorate-treated cotton seeds planted in soil in the greenhouse resulted in 92, 88, 94, and 76% emergence, respectively. The plants grown from intact seeds, both treated and untreated, appeared normal; however, those from the dehulled seeds were stunted and burned, the ones from phorate-treated seeds more severely than those from untreated seeds.

Field Experiment.—The amounts of radioactivity (expressed as phorate-equivalents) found in plants grown

Table 3.—Absorption and translocation of P^{32}-labeled phorate by cotton seeds and plants following treatment with 4 mg. per seed. Seeds were planted in sand in the greenhouse.[a]

Planting to Harvest (days)	Average Micrograms of Phorate-Equivalents Found in—						Total Phorate-Equivalents per Plant[b] (μg.)	Per Cent of Phorate Absorbed[b]
	Seed Coat	Embryo	Radicle or Root	Cotyledons	True Leaves	Stem		
1	6	11	—	—	—	—	11	0.3
2	6	28	8	—	—	—	36	.9
3	10	42	13	—	—	—	55	1.3
4	15	—	12	20	—	—	32	1.2
7	14	—	17	59	—	—	76	1.9
7	—	—	—	—	—	—	66	1.6
14	—	—	—	—	—	—	66	1.6
21	—	—	4	45[c]	2	3	54	1.2
28	—	—	2	43[c]	7	5	57	1.3
35	—	—	2	49[d]	13	6	70	1.7

[a] Each figure is the average of 5 to 10 seeds or plants.
[b] Excluding phorate in seed coat.
[c] 65% extractable with chloroform.
[d] 54% extractable with chloroform.

from phorate-treated seeds planted in soil are shown in table 4. These data demonstrate that very little phorate was absorbed after 1 week. Sixty-four per cent of the seeds emerged. This rather poor stand probably was caused by the lack of adequate moisture during the first 10 days after planting.

DISCUSSION.—Experiments with dehulled seeds revealed that relatively large amounts of phorate in the embryo did not inhibit germination, even at 65° F. Germination of treated intact seeds, which contained much less phorate than treated dehulled seeds, was inhibited or slowed down at all experimental temperatures. These data indicate that there is some relationship between the seed coat and germination. The presence of seed coats reduced the germination and growth of the radicles of untreated intact seeds when compared with untreated dehulled seeds. The phorate treatment appeared to magnify this reduced germination and radicle growth of the intact seeds. The seeds which were on damp filter paper were completely covered with a thin film of water. This film in conjunction with the seed coat may have reduced gaseous exchange of the respiring embryo enough to inhibit germination of some seeds, particularly those of poor quality. The removal of the seed coats would eliminate this possible difficulty. The pronounced reduction in germination of the treated intact seeds could have been caused by the phytotoxicity of the insecticide coupled with a reduction of gaseous exchange of the respiring seeds. A second possible explanation of the reduced germination of phorate-treated intact seeds is that the insecticide merely reduced the vigor of the embryo and resulted in its inability to split the seed coat and successfully germinate. A third possible explanation is that a germination inhibitor was formed by the action of phorate on the seed coat. The first explanation cited appears most plausible. The reduced emergence of treated dehulled seeds planted in soil in the greenhouse, when compared with untreated dehulled seeds, suggests that phorate causes reduced emergence by adversely affecting the seeds after they initiate germination and before the seedlings emerge.

The seed coat reduced the absorption of inorganic P^{32} only during the initial 24-hour germinating period. This reduction may have been entirely because less water was absorbed by the intact cotton seeds than by the dehulled seeds. Since the P^{32} was completely soluble in water, it may have been carried into the embryo by the water.

Linskens (1952) reported that lupine seeds exposed to a 10% parathion emulsion absorbed less water than the controls. However, concentrations of 1 to 0.00001% of parathion caused an increase in water absorption from 2 to 12 hours after exposure. A cotton seed treated with 4 mg. of phorate which absorbed 100 mg. of water would be exposed to a theoretical phorate concentration of 4%, assuming that all of the toxicant was in solution. This theoretical concentration approaches the 10% concentration used by Linskens (1952). It is reasonable to assume that the same phenomena occurred in his experiments as in ours. He also found that mustard seeds gave results similar to those of lupine. However, parathion caused no influence on water absorption by maize and common wheat seeds.

The lack of difference in the rates of respiration of treated and untreated cotton seeds indicates that phorate does not interfere with normal metabolic processes. However, since only seeds which were definitely germinating were used, further work might demonstrate a difference during the first 24 hours of germination.

The data presented here show that seed treatment is an inefficient method of applying phorate. Phorate is absorbed readily by treated cotton seeds only during the first 3 to 4 days after planting. The most rapid absorption

Table 4.—Phorate-equivalents found in plants grown from seeds treated with 4 mg. of P^{32}-labeled phorate and planted in a small field plot.[a]

Planting to Harvest (weeks)	Phorate-Equivalents Absorbed per Plant	
	Micrograms	Percentage
1	173	4.3
2	147	3.7
3	213	5.3
4	187	4.7
6	214	6.0

[a] An average of five plants.

takes place between 24 and 72 hours after planting. This coincides with the splitting of the seed coat and initial growth of the radicle.

The finding that less phorate was absorbed by seeds planted in sand than in soil was not expected. Tietz (1954) showed that demeton, applied as a substrate drench, was absorbed more rapidly by plants grown in sand than in soil. The probable explanation of this apparent discrepancy is that considerable phorate was leached away from the treated seeds in the sand experiment whereas little was leached in the soil experiment because of drier conditions. Also, phorate is relatively short-lived in nutrient solutions (Hacskaylo *et al.* unpublished data) and the hydrolytic products may not be absorbed by the seeds as readily as phorate.

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Effects of Seasonal Rainfall on Range Condition and Grasshopper Population, San Carlos Apache Indian Reservation, Arizona[1]

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ABSTRACT

From 1953 through 1960 a study was made on the San Carlos Apache Indian Reservation to determine the effects of fall and winter precipitation and the response of range plants to weather conditions on populations of spring-hatching grasshoppers. Four subareas of Ash Creek Flat with sparse-to-moderate short-grass associations were used. A rain gauge was located in each subarea. Vegetation surveys were made in spring and summer and grasshopper counts were made biweekly. Results indicate that rainfall from October 1 to March 31 had a direct effect on vegetation and an indirect effect on grasshopper populations. In two 2-year periods (1953–54 and 1957–58) with good rainfall, the spring vegetation was abundant and grasshoppers increased. In the following dry springs of 1955 and 1959 annual weedy vegetation was sparse and stunted. Grasshopper populations were high but decreased drastically by summer. Populations were low in the spring seasons of 1956 and 1960 regardless of range conditions.

On some native rangelands in Arizona grasshoppers are of economic importance periodically, rather than from year to year. Most of the species hatch either in spring or summer. Dominant spring-group species are *Melanoplus bilituratus* (Walker) and *Aulocara elliotti* (Thos.). Less important are *M. cuneatus* Scudder, *Hadrotettix trifasciatus* (Say), and *Psoloessa delicatula* (Scudder).

Spring-group species are of greater economic importance and have more frequent outbreaks than the summer-hatching ones. From surveys in east-central and southeastern Arizona, it has been found that spring-group species almost invariably occur on sparse short-grass areas of intermountain rangeland at elevations of about 5,000 feet.

Several investigators have attempted to correlate grasshopper populations and rainfall. Others have discussed vegetation, particularly specific host plants. However, little has been written about the combined effect of these factors on grasshopper abundance. Clark (1949) reported that in Australia the effect of temperature and moisture on plant growth and thus on populations of Acrididae was both direct and indirect. Anderson & Wright (1952) speculated on how climate produced structural changes in vegetation which might affect the physical habitats of grasshoppers. Nerney (1958) reported differences in populations in Arizona which appeared to be caused primarily by the condition of the short-grass rangeland. A continuation of these studies has been made to evaluate the relationship of rainfall on range condition and grasshopper populations. Data obtained through 1960 are presented herein.

The San Carlos Apache Indian Reservation is located in Pinal, Gila, and Graham Counties in east-central Arizona. The northwest area of Ash Creek Flat rangeland (elevation about 5,000 feet) was used in these investigations.

The vegetation was primarily blue-grama, *Bouteloua gracilis* (H.B.K.) Lag., or a mixture of blue-grama and curly mesquite, *Hilaria belangeri* (Steud.) Nash. Of less

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importance were bunch grass pastures of tobosa, *Hilaria mutica* (Buckl.) Benth., and squirrel-tail, *Sitanion hystrix* (Nutt.) J.G. Smith. Some of the many other perennial grasses and perennial forbs present, relatively unimportant because of their sparsity or local environment, were:

Common Name	*Scientific Name*
Grasses:	
Three-awn	*Aristida* spp.
Sand-dropseed	*Sporobolus cryptandrus* (Torr.) Gray
Side-oats grama	*Bouteloua curtipendula* (Michx.) Torr.
Ring muhley	*Muhlenbergia torreyi* (Kunth) Hitchc.
Vine mesquite	*Panicum obtusum* H.B.K.
Desert saltgrass	*Distichlis stricta* (Torr.) Rydb.
Love grass	*Eragrostis* spp.
Deergrass	*Muhlenbergia rigens* (Benth.) Hitchc.
Forbs:	
Wild-buckwheat	*Eriogonum* spp.
Globe-mallow	*Sphaeralcea* spp.
Wafer parsnip	*Cymopterus fendleri* Gray
Snake-weed	*Gutierrezia californica* (DC.) Torr & Gray
Mountain dandelion	*Agoseris* spp.
Prickle-poppy	*Argemone platyceras* Link & Otto
	Kuhnia rosmarinifolia Vent.
Wolfberry	*Lycium pallidum* Miers
Mariposa	*Calochortus ambiguus* (M.E. Jones) Ownbey
Aster	*Aster hirtifolius* Blake

Woody-type plants in local areas were bear-grass, *Nolina microcarpa* S. Wats., and cholla cacti, *Opuntia* spp. Trees were common in canyons and on hills and slopes of mountains. Conspicuous species on hilly terrain were scrub oaks, and along ravines, tree oaks. Walnut, sycamore, and cottonwood were frequent in canyons. Annual plants were a phase of the vegetation and provided large quantities of forage over short periods in spring and late summer seasons.

The studies were made at four rangeland areas with sparse-to-moderate short-grass stands. These areas were selected on the basis of previous economic grasshopper outbreaks. Area 1 was moderately hilly; area 2 was rolling, open rangeland cut by dry washes and with a gentle north slope; area 3 was level or with a slight northeast slope; and area 4 was on a slight southeast slope.

METHODS.—These have been discussed in detail by Nerney (1960). Surveys were made in April, May, and July to determine the composition, abundance, and changes in vegetative ground cover. The per cent of cover for the four areas was averaged for use in these studies.

Grasshopper counts were made biweekly in each area and the average population recorded on a square-yard basis. The species and their relative abundance were determined from sweep-net collections of 100 to 300 individuals.

In 1953, rainfall was measured at the Ash Creek ranch headquarters. During 1954–55, two additional rain gauges were maintained in the vicinity of the study areas. At the beginning of 1956 a rain gauge was available on each area. The totals used are the averages from all the gauges.

HABITATS AND HABITS.—A brief discussion of the habitats and habits of these grasshoppers is presented to show their relationship to and interdependence on food plant abundance. Habitats of leading species were not associated with all vegetation and soil types found. Sparse stands of a blue grama and curly-mesquite mixture seasonally dominated by low-growing annuals were preferred habitats of *Melanoplus bilituratus*, *M. cuneatus*, *Aulocara elliotti*, and *Hadrotettix trifasciatus*, whereas *Psoloessa delicatula* preferred moderate-to-good short-grass stands with a thin growth of seed stalks and a sparse understory of annual plants. Sandy and sandy-loam soils were preferred to heavy clay soils. Egg-laying sites of *bilituratus*, *cuneatus*, and *trifasciatus* were mostly in soil beneath shrubs and tall weeds, around cow chips or rocks, and in grass sod. *A. elliotti* selected the open bare soil between areas of sod, and *delicatula* used soil sites next to the sod. These sites were usually located near their food plants.

Eggs were laid during the summer and those of *elliotti*, *bilituratus*, and *cuneatus* hatched between March 12 and May 12. Hatching of *trifasciatus* occurred between April 10 and May 20. Peaks were reached 10 to 15 days after hatching began and nymphal growth required about 50 days. In a warm dry season hatching began about 10 days earlier than normal. *M. bilituratus* usually had a second brood resulting from a partial hatch and these were present from August to early winter. Hatching of *delicatula* occurred during August and the late-instar nymphs overwintered. By mid-April, adults were appearing.

In early spring, nymphs were sheltered from inclement weather by low-growing plants, litter, or cow chips and became immobile at an air temperature of 50° F. and soil-surface temperature of 55°. On days with slight wind and rising temperatures (air, 58° and soil-surface, 71°), they crawled to bare-soil openings. When the air temperature reached 72°, they began to feed. In early May, nymphs and callow adults of *bilituratus*, *cuneatus*, and *elliotti* were slightly active with both air and soil-surface readings at 60°. They started to feed at air and soil-surface temperatures of 72° and 63°, respectively. Activity was increased with higher temperatures. In years with high populations, migrations occurred when air readings ranged from 66° to 82° with corresponding soil-surface temperatures of 82° to 109°. Migrants stopped to roost with soil temperatures of 110° to 134°. *M. bilituratus* and *cuneatus* climbed tall plants and posts, whereas *elliotti* roosted on grass sod, cow chips, and rocks. Migrations were variable in length, time period, and direction of movement. They began, both in wet and dry years, in abundantly populated areas from which the grasshoppers traveled 1 to 7 miles. During May most nymphal migrants became adults with *bilituratus* the most active. When soil temperatures were high, some flew into nearby trees to feed and roost. In dry seasons these grasshoppers made short flights, whereas most *cuneatus* and *elliotti* stayed in their preferred habitats. No mass flights were observed. In years with low populations and comparable temperatures there were no mass movements and individuals roosted on grass sod and open soil shaded by low-growing plants. *H. trifasciatus*, a slow-moving grasshopper, was usually seen on or near the ground, although a few adults climbed plants to escape the high ground temperature. The habits of overwintering *delicatula* nymphs were similar to those of the early hatching species, although they remained within their preferred grass habitats. The adults roosted on grass sod or on open, shaded soil during periods of high soil temperatures. Populations of these overwintering grasshoppers were low and fairly constant from year to year.

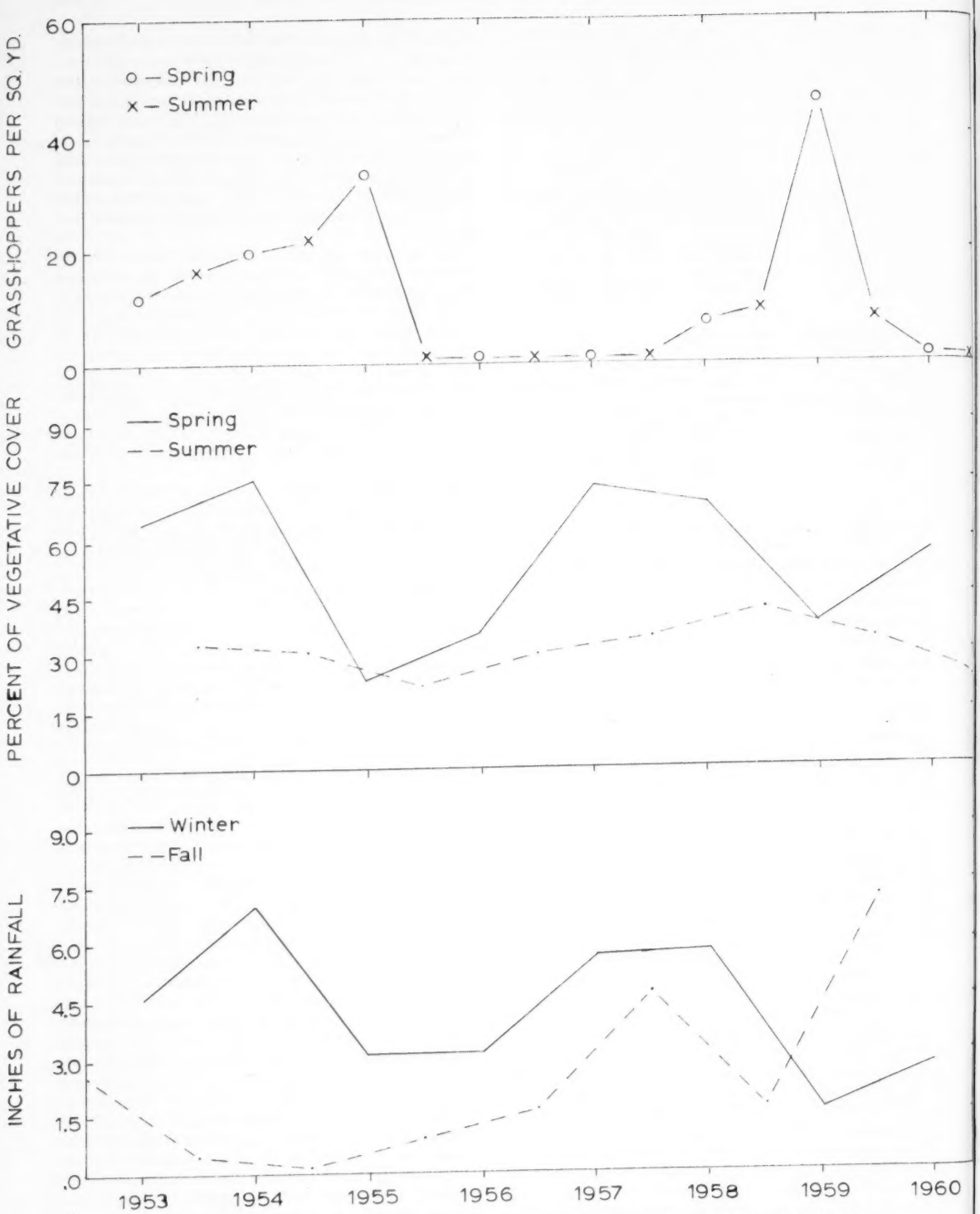

FIG. 1.—Precipitation, vegetative cover, and grasshopper populations in seasons indicated. Note: Rainfall, fall (October 1-December 31); winter, (January 1-March 31).

Table 1.—Effects of seasonal rainfall on vegetative ground cover and grasshopper populations, San Carlos Indian Reservation, Ariz. 1953–60.

Year	Rainfall (inches)			Year	Per Cent of Vegetative Cover			Average Grasshopper Population per Square Yard	
	Oct.–Dec.	Jan.–Mar.	Total		April	May	July	April–May	June–July
1952[a]–53	2.59	4.50	7.09	1953	66	66	35	12.0	16.0
1953–54	0.50	6.95	7.45	1954	76	76	33	20.0	21.0
1954–55[a]	.20	3.08	3.28	1955	46	24	26	33.0	0.6
1955–56	1.16	3.10	4.26	1956	42	35	31	0.2	.4
1956–57	1.57	5.57	7.14	1957	84	73	35	.8	.9
1957–58	4.81	5.73	10.54	1958	—	69	39	6.0	8.0
1958–59	1.71	1.53	3.24	1959	55	38	33	46.0	2.0
1959–60	7.21	2.86	10.07	1960	66	58	24	0.7	0.6

[a] In 1952 and 1955 grasshoppers were controlled with an aldrin spray applied by aircraft.

DISCUSSION AND RESULTS.—Table 1 shows seasonal precipitation, per cent of vegetative cover, and grasshopper populations during the period 1953–60. The precipitation was totaled for October 1-December 31 and January 1-March 31 of consecutive years rather than on a yearly basis. Early spring soil-moisture conditions were wet in 1953, 1954, 1957, 1958, and 1960 with the total fall and winter precipitation ranging from 7.09 to 10.54 inches. In 1955, 1956, and 1959, conditions were dry with a range of 3.24 to 4.26 inches. Rainfall during the spring months of April, May, and June was very light in both wet and dry seasons.

Early season conditions of soil moisture have a direct effect on the per cent of vegetative ground cover during the spring months. In wet seasons the annual vegetation is abundant on sparse short-grass rangeland. The plants are mostly winter and spring annuals, predominantly alfilaria (*Erodium cicutarium* (L.) L'Her.). On fair-to-good grassland, *Gilia gracilis* (Dougl.) Hook., a short-lived winter annual, is most abundant. The vegetation remains succulent throughout the spring and early summer although many annuals mature and give way to bare soil by early June. In dry seasons there is a sparse and stunted growth of weedy plants, mostly winter annuals. These plants and the top growth of weedy perennials are short-lived. By mid-May, vegetation may decrease as much as 60% and the moisture content of perennials becomes very low. Early and mid-summer vegetative cover remains fairly constant regardless of spring or early summer rainfall. During this period, vegetation is composed primarily of perennials that are not as severely affected by adverse early moisture conditions as the annuals which comprise the bulk of the spring vegetation. Summer rains are important, however, in maintaining the perennial plant cover and for germination and growth of annuals in late summer.

Whereas the effects of early rainfall are direct and visible on spring vegetation, those on the grasshopper population are delayed. In early May 1952 and late May 1955 the grasshopper populations were directly affected by an insecticide applied by aircraft; however, in 1952 only a portion of the area was treated. The leading species were *Melanoplus bilituratus* in 1953, *Aulocara elliotti* during 1954–55, and *M. bilituratus* again during 1956–60. Table 1 shows that in the years with early rainfall, 1953–54, spring vegetation was good and grasshopper populations increased. In 1955, a year with dry, early moisture conditions, the spring vegetation was sparse, but the early hatch of grasshoppers was high (33 per square yard). However, owing to the lack of suitable forage, the population had declined 51% by late May, and by summer, after the insecticide application, to less than 1 per square yard. In 1956, a dry year with poor spring vegetation, populations remained low. There were good early moisture and subsequently good early range conditions in 1957 and 1958. In each of these years the grasshoppers gradually increased in numbers and in 1959, notwithstanding low early moisture and poor spring range conditions, the population reached 46 per square yard. As in 1955, the population decreased drastically by summer, owing to poor range conditions caused by deficient early moisture. This decline is illustrated in figure 1 where the fall-winter season precipitation, spring vegetation, and spring and summer grasshopper populations for the period 1953–60 are graphed.

The data in table 1 and figure 1 indicate quite strongly that early rainfall is important to the development of the grasshopper population in areas of Arizona, but only through its effect on the early vegetative cover. Should these indications be borne out by further observations through periods of wet and dry springs, they should aid in range-management programs and in better timing of chemical-control programs in which grasshoppers are involved. In years with dry spring seasons, it may be possible to eliminate control programs even though the spring populations are high. Damage to perennial grass stands would be lessened if the infested areas could be deferred from livestock grazing during the dry seasons.

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Laboratory Tests of Residues of Organophosphorus Compounds against House Flies[1]

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ABSTRACT

Laboratory tests were conducted on the knockdown, kill, and duration of residual effectiveness of 22 organophosphorus compounds against insecticide-susceptible female house flies (*Musca domestica* L.) by confining the insects on plywood panels sprayed with acetone solutions of the materials. As fresh treatments at 50 mg./sq. ft. the most rapidly acting and toxic compounds were DDVP, Dibrom® (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate), Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate), parathion, and ronnel.

In tests on the duration of effectiveness the materials were run alone at 50 mg./sq. ft. and aged outdoors. Ronnel was the most effective in the one experiment in which 1.83 inches of rainfall were recorded, whereas dimethoate was the most effective in another in which only 0.03 inch of rain was recorded. Piperonyl butoxide prolonged the effectiveness of some compounds but inhibited the effects of others.

Many publications have appeared in literature on the toxicity of organophosphorus compounds to house flies, *Musca domestica* L. Although no complete compilation has been made and kept up to date, much helpful information and many good references can be found in the Handbook of Toxicology (Negherbon 1959). In general, most laboratory toxicity measurements have been made by topical application, contact sprays, baits, or by exposure of the flies to films of the insecticide deposited by different methods on glass, wood, or other surfaces.

In the residue tests the tendency has been to expose the flies for short periods to heavy insecticide deposits. Studies were therefore undertaken to determine the minimum lethal and sublethal levels of fresh spray deposits of several compounds and to obtain data on their speed of action, as well as on the duration of their residual effectiveness under practical exposure conditions.

MATERIALS AND METHODS.—The materials selected for tests included those presently recommended for use in the control of house flies, some known to be highly toxic but not recommended, and several newer or less-known compounds. Fresh solutions prepared from the technical grade of material were used in all experiments. The compounds having no accepted common names are listed as follows:

Bayer 22408—*O,O*-diethyl *O*-naphthalimido phosphorothioate
Bayer 29493—*O,O*-dimethyl *O*-(4-methylthio-*m*-tolyl) phosphorothioate
Bayer 37342—*O,O*-dimethyl *O*-(3,5-dimethyl-4-methylthiophenyl) phosphorothioate
Butonate—Dimethyl 1-butyryloxy-2,2,2-trichloroethyl phosphonate
Co-Ral®—*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate
Delnav®-2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate) 68%–70% and related materials
Diazinon®—*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate
Dibrom®—1,2-dibromo-2,2-dichloroethyl dimethyl phosphate
Dipterex®—dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate
Dow ET-15—*O*-methyl *O*-2,4,5-trichlorophenyl phosphoramidothioate
GC 3582—Diethyl-1-(2,5-dichlorophenyl)-2,2-dichlorovinyl phosphate
GC 3583—Diethyl-1-(2,5-dichlorophenyl)-2-chlorovinyl phosphate
GC 4072—Diethyl-1-(2,4-dichlorophenyl)-2-chlorovinyl phosphate
Geigy 30494—*O,O*-dimethyl *S*-(2,5-dichlorophenylthio)-methyl phosphorodithioate
Hercules 4580—*S*-ethoxycarbonylmethyl *O,O*-diethylphosphorodithioate
Ruelene®—4-*tert*-butyl-2-chlorophenyl methyl methylphosphoramidate
Shell SD-3562—Dimethyl 1-(dimethylcarbamoyl)-1-propen-2-yl phosphate

The materials were sprayed on 6-×6-×¼-inch untreated (not waterproofed) plywood panels as freshly prepared acetone solutions. They were applied with a DeVilbiss atomizer at approximately 6 p.s.i. at the rate of 5 milliliters per panel. In the tests involving initial toxicity and knockdown the treatments were allowed to dry for about 1 hour, usually under a fume hood, before being put into test.

Twenty insecticide-susceptible female flies were used per treated panel. Flies from 3 to 5 days old were used in the tests on duration of residual effectiveness and those from 3 to 4 days old in all others. They were knocked down with carbon dioxide, counted, and placed on clean panels. Full recovery from the carbon dioxide was allowed before they were transferred to treated ones. During the tests the flies were fed a 10% sugar-water solution. A piece of cotton in a metal planchet was moistened with the solution and placed under a petri dish to confine the flies on the treated surfaces. A small piece of glass of standard-slide thickness was placed under the edge of the dishes to allow for aeration. The temperature and relative humidity during the tests were from 78° to 80° F. and 60% to 65%, respectively. Knockdown and mortality were recorded after 24 hours of continuous contact with the treated surfaces.

In establishing the initial toxicity levels, three to six tests were made with each material at each treatment rate. The materials were not all tested at the same time; hence the data are not directly comparable. However, in the two tests made on knockdown the materials were all tested at the same time against the same lot of flies.

In the experiments on duration of residual effectiveness the materials were tested at 50 mg./sq.ft. alone and in combination with 250 mg./sq.ft. of piperonyl butoxide. The treatments were run in duplicate; two panels were treated with the compounds alone and two with the compounds plus butoxide. They were aged outside with full exposure to weather conditions, and were suspended from

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Table 1.—Toxicity of residues, after 24 hours, of organophosphorus compounds tested as fresh spray treatments on plywood panels against susceptible 3-to-4-day-old female house flies.[a]

	PER CENT KNOCKDOWN (KD) AND MORTALITY (M)[b] AT INDICATED MG./SQ. FT.																			
	0.01		0.03		0.06		0.1		0.3		0.6		1		3		6		10	
INSECTICIDE	KD	M	KD	M	KD	M	KD	M	KD	M	KD	M	KD	M	KD	M	KD	M	KD	M
DDVP	15	2	80	60	100	100														
Diazinon			8	3	46	38	100	88	100	100										
Dibrom			7	3	73	53	85	82	100	100										
Parathion			0	0	12	5	75	34	100	100										
Ronnel			0	0	15	10	63	48	100	100										
Bayer 29493					0	0	15	10	73	59	100	100								
Dimethoate					0	0	35	20	62	42	98	98	100	100						
Herc. 4580					3	3	28	20	40	27	98	53	100	100						
Bayer 37342							0	0	47	20	83	68	100	100						
GC 4072									0	0	27	10	90	73	100	100				
GC 3582									0	0	22	12	84	48	100	100				
Dow ET-15									5	0	40	30	78	73	100	100				
GC 3583											0	0	40	22	100	100				
CO-Ral											3	3	8	6	100	88	100	100		
Bayer 22408											10	0	30	5	100	80	100	100		
Butonate													7	3	65	60	100	100		
Malathion													0	0	68	48	100	100		
Delnav													7	3	43	23	100	75	100	100
Dipterex													23	19	38	30	78	75	100	100
Geigy-30494													0	0	25	13	73	45	98	73
Shell SD-3562													0	0	23	8	65	43	87	62
Ruelene																	0	0	15	8

[a] An average of 3 to 6 tests; 20 flies/test.
[b] No knockdown or mortality in the controls.

an unshaded wire fence with the treated surfaces facing south so as to receive direct sunlight.

RESULTS AND DISCUSSION.—The data on the toxicity of the 22 compounds tested as fresh spray treatments are presented in table 1. DDVP was the most toxic compound studied and caused 60% and 100% mortality of the flies at 0.03 and 0.06 mg./sq.ft., respectively. It was two to three times as toxic as the next most effective materials—Diazinon, Dibrom, parathion, or ronnel—and approximately 200 times as toxic as Dipterex.

In the tests on knockdown at 50 mg./sq.ft. (table 2) DDVP was also the most rapidly acting compound, causing 50% and 100% knockdown in 3 and 5 minutes, respectively. It was followed in order by Dibrom, Diazinon, Hercules 4580, parathion, and ronnel. The materials followed this same order with respect to toxicity (table 1), except for Hercules 4580. Some of the better-known materials, such as malathion and Dipterex, were relatively slow in action, and 3 to 5 hours were required for complete knockdown of the flies.

The data on residual effectiveness of the materials are presented in table 3. Experiment 1 was started on May 19 and experiment 2 on May 31. In the first experiment five of the materials—Co-Ral, dimethoate, Dipterex, malathion, and Bayer 29493—failed completely after 1 week of aging. Only three compounds—ronnel, parathion, and Diazinon—caused complete kill of the flies alone after 2 weeks of aging. Piperonyl butoxide prolonged the life of some of the compounds, such as GC 4072, Bayer 37342, and Diazinon, but seemed to inhibit the action of Bayer 22408 and parathion. The increased life of some of the compounds by piperonyl butoxide was not unexpected since similar action with phosphorus compounds has been shown by other workers (Eddy *et al.* 1954, and Hoffman *et al.* 1954). Many of the compounds, especially dimethoate, Bayer 29493, and GC 4072, gave higher kills in the second experiment. Here, dimethoate proved to be the

Table 2.—Knockdown time (KD) required for different insecticides tested as fresh treatments on plywood panels at 50 mg./sq. ft. against susceptible 3-to-4-day-old female house flies.[a,b]

	NUMBER OF MINUTES FOR—	
INSECTICIDE	50% KD	100% KD
DDVP	3	5
Dibrom	7	10
Diazinon	13	16
Hercules 4580	23	26
Parathion	38	46
Ronnel	39	47
Gen. Chem. 4072	47	63
Bayer 29493	50	61
Butonate	53	60
Gen. Chem. 3582	53	72
Co-Ral	70	86
Bayer 37342	72	120
Dow ET-15	74	103
Gen. Chem. 3583	78	141
Bayer 22408	84	103
Geigy 30494	85	132
Dimethoate	94	130
Malathion	121	170
Delnav	143	194
Shell SD 3562	251	—[c]
Dipterex	271	300
Ruelene	290	—[c]

[a] No knockdown or mortality in the controls.
[b] Average of 2 to 3 tests.
[c] 8 to 24 hours.

Table 3.—Residual effectiveness of insecticides alone and in combination with piperonyl butoxide against house flies.[a]

Insecticide	Average 24-Hour Mortality (%)[b] After Weeks Shown											
	1		2		3		4		5		6	
	A[c]	B[c]	A	B	A	B	A	B	A	B	A	B
Experiment 1												
Co-Ral	0	0										
Dimethoate	0	0										
Dipterex	0	0										
Malathion	0	0										
Bayer 29493	0	0										
Hercules 4580	35	92	0	5								
Bayer 22408	63	5	0	5								
GC 4072	65	100	75	95	25	72	0	50	—	75	—	37[d]
Delnav	67	85	75	37	25	0						
DDVP	77	100	12	17								
Bayer 37342	100	100	70	0	7	—						
Dibrom	100	100	70	90	60	20	0	7				
Ronnel	100	100	100	100	100	100	100	100	97	100	52[e]	97[f]
Parathion	100	100	100	75	77	5	77	—				
Diazinon	100	100	100	100	20	95	7	90				
Mean temp. (° F.)	45		57		60		61		55		62	
Avg. rainfall (in.)	1.4		0.2		0		0.2		0.03		0	
Experiment 2												
Co-Ral	0	0										
Dimethoate	100	100	100	100	100	100	100	100	100	100	100	100
Dipterex	0	0										
Malathion	93	95	52	90	0	15						
Bayer 29493	100	100	90	95	30	90	65	87	55	50	22	12
Hercules 4580	22	45										
Bayer 22408	77	2	0	1								
GC 4072	100	100	100	100	90	100	97	100	92	100	17	77
Delnav	27	22										
DDVP	100	100	97	97	0	10						
Bayer 37342	100	100	65	90	5	30						
Dibrom	100	100	100	100	57	90	10	77	—	10		
Ronnel	100	100	95	100	80	100	22	30	5	5		
Parathion	100	7	90	0	—	0						
Diazinon	100	100	95	100	60	87	7	7				
Mean temp. (° F.)	63		60		55		61		62		67	
Avg. rainfall (in.)	0		0		0.03		0		0		0	

[a] Treatments sprayed on plywood panels and aged out-of-doors.
[b] No mortality in the controls.
[c] A, alone; B, plus piperonyl butoxide.
[d][e][f] —10%, 0%, and 70% mortality after 7 weeks, respectively.

most effective and caused complete kill of flies through 6 weeks, whereas in experiment 1 it caused no kill after 1 week of aging. The influence of piperonyl butoxide was about the same in both experiments.

It should be noted that 1.83 inches of rainfall was recorded during the first experiment, whereas only 0.03 inch was recorded during the second test. In view of this fact and because the temperature was generally higher in the second test, one would judge that rainfall rather than higher temperatures was responsible for the difference in performance of the materials in the two experiments.

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Studies on Parathion Metabolism in Normal and Parathion-Resistant House Flies[1]

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ABSTRACT

The absorption, detoxication, and excretion of parathion and para-oxon were studied in susceptible and parathion-resistant strains of the house fly (*Musca domestica* L.) by radiometric techniques. No differences in the rate of absorption of the toxicants were noted between susceptible and resistant flies. Both insecticides were rapidly detoxified by the flies *in vivo*, with the metabolism being more rapid in the resistant strain. Differences in the rate of excretion of parathion and/or its metabolites were not great, but para-oxon and/or its metabolites were excreted much more rapidly by flies of the resistant strain.

Several previous studies on the metabolism of organophosphorus compounds in normal and resistant insects have been reported. Lord & Solly (1956) compared the metabolism of para-oxon in a normal and in a two-fold resistant strain of house flies (*Musca domestica* L.) and found no apparent difference in the rate of detoxication between the strains. Oppenoorth (1958) showed that in normal, slightly tolerant, and resistant strains of flies, the susceptibilities to parathion were inversely related to the rate at which they metabolized para-oxon. March (1959) reported that a highly malathion-resistant strain of flies metabolized mala-oxon (S-[1,2-bis(ethoxycarbonyl) ethyl] *O,O*-dimethyl phosphorothiolate) twice as fast as a susceptible strain.

In the studies described above, measurements of cholinesterase (ChE) activity were employed in determining the fate of the insecticides. The much greater inhibitory power of the oxygen analogs of phosphorothioate insecticides precluded the possibility of making direct enzymatic measurements on the fate of the parent compounds, and so their metabolic fate was not directly determined.

In order to study the metabolic fate of a phosphorothioate insecticide itself, as well as of its oxygen analog, the use of techniques involving nonenzymatic methods of detection is necessary. We obtained a sample of radioactive parathion of extremely high specific activity from entomologists at our Orlando, Fla., laboratory. The high level of radioactivity enabled us to make direct measurements on the fate of parathion in resistant and normal house flies at sublethal dosages by means of radiometric techniques. A part of the labeled parathion was converted to para-oxon and comparable studies were made on the fate of this material. Data are given on the toxicity, absorption, excretion, and detoxication of both compounds in a normal and in a parathion-resistant strain of the house fly.

MATERIALS AND METHODS.—*Insecticides.*—At the time of receipt, the radioactive parathion had an activity of 6.7×10^4 c.p.m./μg. as determined by a proportional counter and a radiochemical purity of 98% based on several paper-chromatographic techniques. Radioactivity in all samples was determined with either a windowless gas-flow proportional counter or an automatic Geiger counter. All experiments described in the paper were performed within 4 half lives of the labeled phosphorus, so that the minimum activity level was about 4,000 c.p.m./μg.

A portion of the labeled parathion was converted to para-oxon by means of the nitric acid oxidation method of Johnson (1955). A sample of the final product, extracted with ether, was analyzed by the reversed-phase paper-chromatographic system of Metcalf & March (1953) and found to contain approximately 25% of para-oxon, 70% of parathion, and small amounts of several other materials. The ether extract was then chromatographed on alumina (Krueger *et al.* 1959), the parathion being eluted with benzene and para-oxon with chloroform. The chloroform fraction was rechromatographed on alumina and its purity then determined by paper chromatography. The purity of the final preparation of para-oxon was 97% and the yield was 600 μg., or 2.4%. It may be noted that whereas parathion was quantitatively eluted from alumina with benzene, para-oxon was eluted only partially with chloroform, with at least half the radioactivity left on the column both times. Attempts to elute the radioactivity remaining on the column with other solvents were unsuccessful. Nevertheless this method was used as it gave a highly purified product.

Test Insects.—Two- to four-day-old female house flies were used in the experiments described here. Susceptible flies were from the Orlando Regular Colony which has been maintained at this laboratory for several years. Resistant flies were from the Tropical P colony, maintained at Orlando by pressuring with parathion (Wilson *et al.* 1959). All studies in which resistant flies were used were performed on the F_1, F_2, and F_3 generations from pressured parents which were maintained in the laboratory without further exposure to parathion.

Flies were bioassayed with parathion and para-oxon by topical application of the insecticides in 1.117 μl. of acetone to the tip of the abdomen using a micrometer-driven syringe. They were also bioassayed by exposure to insecticide films in pint-sized glass jars, 10 cm. in height, and 7.5 cm. in diameter. The films were prepared by pipetting the requisite amount of insecticide in 1 ml. of acetone into the jars and then rotating the jars on their sides until the solvent was evaporated. Treated insects were held at 80° F. and 70% relative humidity and mortalities were determined 24 hours later.

Insecticide Metabolism.—The fate of both insecticides in normal and parathion-resistant flies was compared at a dosage level for both materials of 0.004 μg./fly, an amount representing less than half that required to produce a 50% mortality in the susceptible flies with either material. In addition, the metabolism of parathion was studied in both

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colonies of flies at a dosage of 0.044 μg./insect. This dosage killed all the normal flies within 4 hours but caused less than a 10% mortality in the resistant strain. Five to 15 flies per sample were treated and each sample was replicated three times for each time interval at which treated insects were sacrificed. Each sample for analysis contained a minimum of 2,000 c.p.m. The flies were kept in the holding room in shell vials lined with filter paper in an attempt to minimize possible reabsorption of any excreted radioactivity.

The rate at which the toxicants were absorbed by normal and parathion-resistant flies following topical abdominal application of the toxicants was determined. At selected time intervals flies were anesthetized with carbon dioxide, placed on a glass-wool plug in a funnel, and the unabsorbed insecticide removed by rinsing repeatedly with small aliquots of acetone. The acetone rinses were pooled in planchets and counted to determine the radioactivity remaining externally on the insects.

Detoxication studies were performed on the same flies used in the absorption studies. After removing unabsorbed insecticide, the samples of flies from each time interval were combined, frozen, and then macerated with sand and water (1 ml. per 5 flies) by means of a mortar and pestle. An equal volume of chloroform was added and the mixture shaken and centrifuged. Aliquots of the chloroform (=parathion and/or para-oxon), water (=detoxified insecticide), and the proteinaceous residue were counted.

Excretion of the toxicants was measured in separate studies. Flies were treated as described above and sacrificed by freezing at selected time intervals. Whole flies were then counted directly in planchets to determine the radioactivity still present on and in the flies.

The possible conversion of parathion to para-oxon was not measured by enzymatic methods in this study. Attempts to measure the conversion by alumina or paper chromatography yielded unsatisfactory data and so no information on possible differences in the rate of conversion between the normal and the parathion-resistant flies was obtained.

Attempts were made to characterize the breakdown products of both insecticides utilizing acetonitrile:water, 85:15, paper chromatography (Kaplanis *et al.* 1959). Excreta from the filter-paper liners in which flies were held were extracted overnight with acetone, the solvent evaporated, and the radioactivity spotted on Whatman No. 1 paper. Insufficient radioactivity was obtained to allow for analyses by ion-exchange chromatography, although the metabolites of parathion from insects have been previously characterized by this technique (Plapp & Casida 1958).

Table 1.—Twenty-four hour LD-50's and LD-90's of parathion and para-oxon for susceptible (S) and parathion-resistant (R) 3-day-old female house flies.

	Parathion		Para-oxon	
	S	R	S	R
Bioassay by topical applications of toxicants in acetone—μg./fly				
LD-50	0.012	0.06	0.015	0.05
LD-90	.03	.18	.035	.15
Bioassay by exposure to insecticide residues in glass jars—μg./jar				
LD-50	0.75	8	2	4
LD-90	1.0	16	6	28

Table 2.—Absorption of topically applied parathion and para-oxon by susceptible (S) and parathion-resistant (R) house flies.

Time After Treatment (min.)	Per Cent of Applied Dose Absorbed					
	Para-oxon 0.004 μg./fly		Parathion 0.004 μg./fly		Parathion 0.044 μg./fly	
	S	R	S	R	S	R
5	45	44	40	39	—	—
10	55	60	49	52	—	—
15	67	65	60	61	57	—
20	76	71	65	70	—	—
30	80	75	78	80	78	73
60	88	92	83	85	86	79
120	92	95	92	95	94	95
240	97	98	96	99	98	98

Results.—The data on susceptibility of normal and parathion-resistant flies to parathion and para-oxon are given in table 1. With both materials the apparent "resistance" in the Tropical P colony was greater by the film method than by topical bioassay. Resistance to para-oxon was of the same order of magnitude as resistance to parathion, which indicated, as has been reported previously, that differences in intoxifying factors are apparently not related to resistance.

Both toxicants were rapidly absorbed by susceptible and resistant flies (table 2), para-oxon being absorbed more rapidly than parathion. At a dosage rate of 0.004 μg./fly, the times for a 50% absorption of both materials were 10 minutes or less. No differences were noted between colonies that could explain the resistance present.

Data on the breakdown of the insecticides under *in vivo* conditions are presented in table 3. The figures given represent only radioactivity present in the bodies of treated flies extractable with solvents at the different sampling times and do not include unabsorbed, unextract-

Table 3.—Detoxication of parathion and para-oxon by susceptible (S) and parathion-resistant (R) house flies.

Time After Treatment (min.)	Per Cent of Extractable Radioactivity Partitioning into Water					
	Para-oxon 0.004 μg./fly		Parathion 0.004 μg./fly		Parathion 0.044 μg./fly	
	S	R	S	R	S	R
5	57	86	—	—	—	—
10	63	78	—	—	—	—
15	59	67	17	32	22	—
20	58	75	—	—	—	—
30	63	84	38	58	27	49
60	80	92	51	58	36	59
120	85	89	58	83	47	73
240	84	90	90	90	70	64
480	96	97	95	96	74	86
720	96	92	100	100	79	89
1,440	92	90	100	100	93	99

able, or excreted radioactivity. The results may be summarized as follows:

1. Para-oxon was rapidly metabolized by flies of both colonies, a minimum of 80% of the absorbed radioactivity being converted to water-soluble products within 1 hour of the time of treatment.

2. The metabolism was faster in the resistant strain, the toxicant being detoxified almost as fast as it was absorbed.

3. Parathion was detoxified more slowly than para-oxon by both colonies of flies, 2 to 4 hours being required to eliminate the insecticide at a sublethal dosage level and even more time required at the higher level.

4. At both dosage levels the resistant flies metabolized parathion more rapidly, which indicated that a more rapid detoxication of the parent phosphorothioate as well as of the toxic oxygen analog is a factor in resistance. Apparently death did not end the metabolism of parathion, since in the normal flies that died within 4 hours of the time of treatment, large additional quantities of the toxicant were destroyed after the insects appeared to be dead.

In addition to the differences already noted in the metabolism of parathion and para-oxon between normal and resistant flies through studying the partitioning properties of the extractable radioactivity, another difference was noted. In the case of para-oxon, significantly larger quantities of the administered radioactivity were bound to the tissues of normal flies, that is, unextractable with water and chloroform for several hours after treatment than was the case with the resistant flies. Much smaller differences were noted with parathion. Data on this phenomenon are summarized in table 4.

Whether the larger amount of radioactivity bound to the tissues in normal flies treated with para-oxon was in the form of insecticide or as breakdown products was not determined. However, it is felt that it probably represents a difference in detoxication rates between the colonies. By 8 hours after treatment quite similar amounts of radioactivity were extractable from homogenates of both colonies. By this time metabolism of the administered toxicant was complete and the remaining tissue-bound radioactivity was probably present as phosphoric acid formed upon complete metabolism of the insecticide. Similar difficulties in extraction of radioactive phosphorus insecticide were noted in a study by Krueger *et al.* (1959).

Table 4.—Ease of extraction of absorbed parathion and para-oxon from homogenates of susceptible (S) and parathion-resistant (R) house flies.

Time After Treatment (min.)	Per Cent of Absorbed Radioactivity Not Extractable					
	Para-oxon 0.004 μg./fly		Parathion 0.004 μg./fly		Parathion 0.044 μg./fly	
	S	R	S	R	S	R
5	56	25	—	—	—	—
10	52	25	—	—	—	—
15	50	25	26	40	38	—
20	58	28	—	—	—	—
30	58	29	39	38	34	25
60	46	28	41	45	43	32
120	42	30	50	42	37	23
240	46	36	55	62	31	27
480	47	40	47	50	30	32
720	47	42	60	52	32	20
1,440	53	44	48	55	27	22

Table 5.—Excretion of parathion and para-oxon by susceptible (S) and parathion-resistant (R) house flies.

Time After Treatment (min.)	Per Cent of Administered Dose Excreted					
	Para-oxon 0.004 μg./fly		Parathion 0.004 μg./fly		Parathion 0.044 μg./fly	
	S	R	S	R	S	R
30	11	11	13	14	0	0
60	14	26	31	15	0	0
120	21	34	43	37	20	27
240	20	28	52	49	26	44
480	22	64	58	58	34	68
720	48	59	63	64	21	75
1,440	44	59	67	73	—	—

Measurements on the excretion of the two insecticides by both colonies of flies are summarized in table 5. The results with para-oxon are in agreement with those obtained in the detoxication studies in that the parathion-resistant flies excreted the administered toxicant at a more rapid rate than did the normal flies. No differences in the rate of excretion were noted with parathion at a dosage nontoxic to both colonies of flies. With flies treated at the higher dosage of parathion, marked differences were noted only after the time of death of the normal flies. Evidence was obtained for the presence of both diethyl phosphoric acid and diethyl phosphorothioic acid as metabolites of parathion, but the amounts of radioactivity present were small and no gross differences between the colonies in the nature of the excretory products were noted.

DISCUSSION.—The results obtained in this study show that under *in vivo* conditions both parathion and para-oxon are readily detoxified by susceptible as well as resistant house flies. With para-oxon the breakdown is especially rapid and is substantially complete within 1 hour of the time of treatment in both susceptible and resistant flies. The data are in substantial agreement with those of Lord & Solly (1956) who found that detoxication of para-oxon was complete within 2 hours.

The results are in agreement with previous reports that more rapid destruction of para-oxon is accomplished by parathion-resistant flies. The data also show that more rapid metabolism of parathion itself occurs and is probably a factor in resistance.

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Laboratory Evaluation of Insecticides against the Adult Horn Fly[1]

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ABSTRACT

In topical applications of insecticides to adult, field-collected horn flies (*Siphona irritans* (L.)) in Oregon, the LD-50 and LD-90 dosages, respectively, in micrograms per fly were: DDT, 0.0058 and 0.019; methoxychlor, 0.025 and 0.072; toxaphene, 0.017 and 0.037; and malathion 0.012 and 0.04. Data are presented on the comparative susceptibility of horn flies, stable flies (*Stomoxys calcitrans* (L.)), and house flies (*Musca domestica* L.) to DDT.

DDT, toxaphene, and methoxychlor have been widely used for the control of the horn fly (*Siphona irritans* (L.)) for about 15 years. Therefore the recent reports of suspected resistance in this species have been anticipated. However, none of the reports have been accompanied by sufficient quantitative data to establish the degree of resistance that has developed, probably because satisfactory test techniques have not been worked out for measuring horn fly susceptibility. Studies were therefore undertaken at the Corvallis, Ore., laboratory to ascertain whether currently used topical and residual test techniques could be modified for susceptibility determinations with the horn fly. Results of these studies are presented in this paper.

MATERIALS AND METHODS.—Both field-collected and laboratory-reared flies were used in the tests. Before testing, the field-collected specimens were held for 2 days on a clean animal confined in a screened stall. The eggs from which flies were reared in the laboratory were obtained by confining the adults in battery jars containing fresh cow manure as an oviposition medium. Development of the immature stages was at 80° F. Emergence was allowed to proceed for 2 days in a screened stall containing a host animal. Topical tests were made on the reared females 4 days after emergence started.

For testing, the flies were removed from the animals with a net and placed in glass jars. A second transfer was made in the laboratory to eliminate any weak or injured individuals. The flies were knocked down with carbon dioxide for counting and/or sexing.

In the topical tests the thorax of each fly was treated with 1 μl. of an acetone solution of the insecticide. The solutions were applied with a micrometer-activated $\frac{1}{4}$-cc. tuberculin syringe equipped with a blunted 26-gauge needle bent to a 90° angle.

Field-collected flies of mixed sexes were used in the residue or knockdown tests. Screen-wire cylinders, $3\frac{1}{2}$ inches in diameter and 8 inches in length, were dipped in 0.5% acetone solutions and aged 24 hours before testing.

Prior to these tests it was found that excellent 24-hour survival of the flies could be obtained when they were fed either sucrose or honey-water solutions. In these tests food was provided by placing cotton pads saturated with a 5% honey-water solution on top of the screen-wire lids of the pint-sized glass holding jars. The temperature and relative humidity during the tests were 74° F. and 72%, respectively. Fly mortality was recorded after 24 hours.

RESULTS AND DISCUSSION.—*Topical Tests.*—The results of topical tests against both laboratory-reared and field-collected flies are presented in table 1. One test, replicated twice, was run with DDT on laboratory-reared flies. The estimated LD-50 and LD-90 values in micrograms per fly were 0.0071 and 0.0265, respectively. The data on field-collected flies were obtained from three collections, with two replicates being run with most of the dosages. Although the values are somewhat lower than for the laboratory-reared specimens, the dosage-mortality curves are similar.

Flies for the methoxychlor tests were also field-collected on three different occasions. For most dosages two replicates were made from each collection. The estimated LD-50 and LD-90 values in micrograms per fly were 0.025 and 0.072, respectively, which make the dosage-mortality curve for methoxychlor approximately parallel to that of DDT.

Flies for the malathion tests were also field-collected on three different occasions. For most dosages two replicates were made from each collection. The estimated LD-50 and LD-90 values in micrograms per fly were 0.012 and 0.04, respectively, which make the dosage-mortality curve for malathion also approximately parallel to that of DDT.

Only one group of field-collected flies was tested with toxaphene. The estimated LD-50 and LD-90 in micrograms per fly were 0.017 and 0.037, respectively. The dosage-mortality curve was considerably steeper for toxaphene than for the other insecticides.

Residue Tests (Knockdown).—In the knockdown tests

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Table 1.—Comparative effectiveness of several insecticides applied topically to reared and wild female horn flies.[a]

Insecticide	Dosage (μg./fly)	Mortality[b] (%)	Micrograms per Fly for— LD-50	LD-90
		Reared flies[c]		
DDT	0.002	6.3	0.0071	0.0265
	.004	25.0		
	.008	65.6		
	.016	75.0		
	.032	90.6		
		Wild flies[d]		
DDT	0.002	16.3	0.0058	0.019
	.004	25.3		
	.008	68.6		
	.016	79.6		
	.032	94.3		
Methoxychlor	.008	15.3	.025	.072
	.016	46.5		
	.032	42.8		
	.064	70.9		
	.096	100		
Toxaphene	.004	26.5	.017	.037
	.008	11.8		
	.016	38.2		
	.032	85.3		
	.064	100		
Malathion	.0025	3	.012	.040
	.005	37.7		
	.01	57.3		
	.02	48.7		
	.04	87.5		
	.06	98.5		

[a] 2 to 6 replications per concentration.
[b] Adjustment by Abbott's formula. With field-collected flies mortality in acetone-treated controls averaged about 10%. No mortality occurred with non-acetone-treated flies, or in any controls of reared flies.
[c] 16 females per replicate.
[d] 20 females per replicate.

run in duplicate with mixed sexes of field-collected flies in which the insecticides were deposited on screen-wire cylinders, methoxychlor had the most rapid action with 100% knockdown in 11 minutes, followed by DDT in 12, malathion in 35, and toxaphene in 118 minutes.

The knockdown times obtained with wettable powders at Kerrville, Texas by Eddy & Graham (1949) were generally shorter than those obtained in these experiments with the technical material. However, the differences in test conditions and insecticide formulation could have accounted for this difference.

Relationship of Size of Fly to Susceptibility.—In some reports on insect susceptibility to insecticides the results are summarized in micrograms of toxicant per gram of insect. Seventeen horn fly males weighed during this investigation averaged 3.38 mg. per fly. Weights of individual females ranged from 5.8 to 10.6 mg. Forty-six randomly selected females averaged 7.95 mg., or about 126 flies per gram. Thus, to obtain LD-50's per gram of female flies for DDT, malathion, toxaphene, and methoxychlor, 0.73, 1.51, 2.14, and 31.5 μg., respectively, of the insecticides would be required. To obtain LD-90's, on the same weight basis, 2.39, 4.66, 5.04, and 9.07 μg. of the insecticides, respectively, would be required. Metcalf (1955) lists the LD-50 for DDT to susceptible house flies as ranging from 8 to 21 μg. per gram of female fly. DDT topical tests with a Corvallis, Oregon and a Nebraska strain of stable flies (*Stomoxys calcitrans* (L.)) gave an average LD-50 and LD-90 for the combined strains of 0.13 and 0.26 μg., respectively, per female fly. On the basis of an average of 38.57 females per gram for these combined strains, the estimated LD-50's and LD-90's were 5.21 and 10.34 μg. of DDT, respectively, per gram of female stable fly. These data would indicate that on a per-gram basis house flies and stable flies are more tolerant of DDT than horn flies.

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SCIENTIFIC NOTES

An Improved Backpack Motor Fan for Suction Sampling of Insect Populations[1]

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The principal objection to the use of the portable air suction machine (Dietrick *et al.* 1959, 1960) for sampling insect populations in alfalfa fields has been the weight of the motor fan unit. Hence, the design and manufacture of a light weight portable motor fan became desirable (fig. 1, W). This has been accomplished by the author in cooperation with the Master Fan Corporation of Los Angeles, California. The backpack mounting enables the operator to have both hands free to manipulate the collecting attachments, thus making it possible for one person to take samples.

The overall dimensions of the new backpack motor fan unit are 15×15×24 inches and the weight is 27 pounds. The air flow capacity is the same as was obtained by the heavier machine (Dietrick *et al.* 1959). A single-cylinder, air-cooled, two-cycle gasoline engine of the type used on lawn mowers is used to turn the light weight fan. The air intake is through an 8-inch opening from the top and the exhaust is straight out the back. The motor is mounted below the fan, thus keeping the center of gravity low for easy balance. The carburator is of the non-float type which

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FIG. 1.—A new backpack vacuum insect collector: W, various attachments; X, square foot area sampler; Y, adapter hose for increased suction; Z, vacuum insect net.

feeds gasoline no matter what the position of the motor is.

A flexible air duct connects the various collecting attachments to the fan intake and is a 5-foot piece of 8-inch diameter, wire-ribbed, canvas air duct. The portion of the air duct which holds the organdie insect collection bag is formed by a fiber glass cylinder. This 13½-inch diameter by 11-inch cylinder is connected to the flexible air duct by a tapering section of plastic-coated nylon. This 18-inch long, nylon section is held open by wire ribs and two metal supports. The entire air duct folds accordian-like into a convenient package for transport.

There are many possible collecting attachments which may be operated from the basic motor fan and flexible air duct unit. The insects are sieved by the organdie bag as near to the collecting head as possible. This bag needs to be large enough and tapered to allow for good air flow after it begins to fill with insects and duff. This arrangement enables the insects to be screened without damage to them.

The square-foot area collecting head is copied after the one used in the previous reference except that it is made from light weight fiber glass material. This cylinder fits inside the attached cylinder on the end of the air duct, and holds the collecting bag in place. The open end of this collecting head cylinder is ringed with small, screen-covered openings. When the collecting head is pressed against the ground, these openings allow for a continuous flow of air which is necessary to gather the insects efficiently and to hold them and the duff firmly in the organdie bag, regardless of the position of the collecting head (fig. 1, X).

The size of the openings of these unconnected cylinders can vary with the size of the plant or the purpose of the collection. Fiber glass cones can expand or decrease the area covered by the open end of the collecting head cylinder. Row crops may be sampled with a long narrow collecting head or by squeezing the fiber glass cylinder to fit the row of plants. Smaller flexible hoses may be adapted to fit inside the collecting bag (fig. 1, Y). Cotton has been sampled by placing cylinders of different size over the plants, increasing the height of the cylinders as the plants grow.

There are limits of plant size where this method of completely covering the plants becomes impractical. A handle may be connected to the collecting cylinder and the unit may be used like a sweep net (fig. 1, Z). This vacuum insect net may be used to brush over the foliage, or placed over entire branches of larger plants or limbs of trees. Citrus, pear, and oak trees have been sampled in this manner.

The backpack insect collector has been used extensively during 1960 for collecting and studying beneficial insects on cotton. It has proved to be most useful in studying and sampling the tiny egg parasites, Trichogrammatidae, and the Mymaridae. A larger proportion of the immature stages, small caterpillars and even eggs, are gathered, giving earlier warning of needed control measures, allowing time to make application before the worms are large enough to cause economic damage. A more complete and accurate estimate of the total insect population may be obtained from samples gathered by this method (Dietrick & Schlinger 1960). The modifications described here make the equipment more flexible and useful in sampling insects and other arthropods on many crops, and could very well become a useful tool for the collection and detection of new species in survey work (foreign or domestic) and in quarantine work.

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Some Shortcuts in Laboratory Rearing of Boll Weevils[1]

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Numerous papers have been written on the laboratory rearing of the boll weevil, *Anthonomus grandis* Boh., by use of artificial diets (Vanderzant & Davich 1958, Vanderzant 1959, Vanderzant *et al.* 1959, Brazzel *et al.* 1959, Earle *et al.* 1959). In all of these papers the rearing involved the use of cotton squares with the leaf bracts removed as an oviposition site. The squares were then dissected by hand and the eggs removed for transplanting into the culture media. Considerable time and labor are required to dissect the squares and this is a major factor in the cost of rearing. Vanderzant & Davich (1958) estimate that an experienced worker could pick out an average of 100 eggs per hour. The method described in this paper has yielded as high as 500 eggs in 10 minutes. A second time-consuming task is the removal of the leaf bracts from the cotton squares. The time used in this operation was reduced by 80% by means of mechanical rollers.

EGG EXTRACTION.—Fresh cotton squares were placed in oviposition jars containing the adult weevils. Pint fruit jars fitted with screen caps were used. No moist sand was used in the jars as suggested by Vanderzant & Davich (1958) as desiccation of the squares was not a problem. Should desiccation become a problem, a pad of moist blotting paper in the bottom of the jar would prevent it. After 24 hours, the weevils were separated from the squares by dumping the entire contents of the jar onto a Number 8, U. S. Bureau of Standards 8″ screen, and gently shaking the screen to dislodge the weevils. The weevils were collected in a pan and returned to the rearing jar. No injury to the adult weevils resulted unless the screen was shaken too vigorously.

The squares were next placed in a Waring Blendor one-half full of water. The blender was run approximately 2 seconds and then the entire contents poured through a nest of 8-inch-diameter screens consisting of the following sizes: U. S. Bureau of Standards Numbers 8, 16, 20, 30, and 40. The material on the top screen (No. 8) is then returned to the blender and ground with water for another 2 seconds. The process is repeated two or three times or until all the squares are broken open and the anthers removed. Normally the ovaries remain intact with only the sepals and immature petals being broken open. If the blender is allowed to run for more than 2 seconds at a time, the dislodged eggs suspended in the water will be destroyed.

After the grinding has been completed, the plant material is washed through the screens by means of a fine jet of water. Each screen is washed thoroughly and then removed from the top of the nest. Special care should be taken when washing the number 20 and 30 screens as the eggs may lodge in the fine plant material. The number 30 screen will retain most of the plant material while the eggs will be caught on the No. 40 screen. At this point the material on the number 40 screen will be about 50% eggs and 50% plant debris by volume. The material is concentrated at one edge of the screen with water and washed into a 250-ml. separatory funnel with 100 ml. of a saturated salt solution. After a few seconds, the plant material will lose water to the salt solution and start to settle, while the eggs being relatively impervious to water loss will float. The salt solution and plant debris are drawn off the bottom until the first of the eggs starts to pass through the stopcock. The salt separation may be repeated if necessary but generally once is all that is needed. Next the

[1] Contribution from the Entomology Department, North Carolina Agricultural Experiment Station, Raleigh. Published with the approval of the Director of Research as Paper No. 1242 of the Journal Series. Accepted for publication November 14, 1960.

Table 1.—Per cent of boll weevil egg hatch and adult emergence by two methods of egg extraction.

Number of	Blender-Salt Extracted	Hand Extracted
Eggs	100	100
Eggs hatched	87	85
Adults	67	69

sterilizing solution (Vanderzant & Davich 1958) is added to the funnel. This causes the eggs to sink while much of the remaining plant material will float or adhere to the sides of the funnel. The surface sterilized eggs are then drawn off the bottom and onto a filter paper in a 1-inch-diameter split buchner funnel. After two rinses with sterile water, the eggs are ready for transplanting to the media. About 5% to 10% plant material will remain with the eggs, but this can easily be discarded as the eggs are planted. Tests made with manually extracted eggs and those extracted by the above procedure showed no difference in the percentage hatching or the number of adults successfully reared from the eggs (table 1).

About 10 minutes is required to obtain the sterilized eggs from a group of squares, and as many as 500 eggs have been obtained from a single group. It should be possible to obtain several thousand eggs in the same length of time if sufficient squares were available. Tests were made to determine the per cent recovery by the blender method as compared with manual extraction. Groups of squares containing eggs were divided into four random subgroups. Two of these subgroups were dissected manually while the other two subgroups were run through the blender. The number of eggs recovered manually was taken as 100% and the number from the blender was compared with this. The highest recovery by the blender was 85% while the lowest was 43%. The average was 62% (table 2). The best recovery of eggs was possible when the squares were very fresh. If any drying of the sepals occurred, they became tough and required additional grinding to break the squares open. When this happened, a higher percentage of the eggs was destroyed by the blender. It has been the experience of the author that the blender extraction works best when more than 100 squares are used in a group. Smaller numbers would probably work equally as well if the blender cup were smaller than the ordinary quart size.

Although there was some loss in the numbers of eggs recovered by the blender method, this was offset by a tremendous saving in time and labor. Additional breeding stock could easily overcome this loss.

LEAF BRACT REMOVAL FROM SQUARES.—The task of removing the leaf bracts from the cotton squares is not nearly as time consuming as the extraction of the eggs from the squares, but as much as 45 minutes to an hour can be spent in this operation if there is a large number of squares needed to have the bracts removed. A simple motor-driven set of rollers (fig. 1) makes it possible to remove the bracts from several hundred squares in about 5 minutes. The rollers are made by cementing $\frac{1}{4}$-inch I.D. rubber tubing, 3″ long, onto $\frac{1}{8}$-inch steel rods. The rollers are then mounted in a frame so that they touch similar to the wringer rolls on a washing machine. The ends of the rollers are connected by gears so that they rotate in opposite directions (the $\frac{1}{4}$-inch-diameter spline gears from an old clock are ideal for this purpose) and one of the shafts is fastened to an electric motor of sufficient power to revolve the rollers rapidly. A metal shield is next mounted on the roller frame with a slot in the shield being located directly over the place where the rollers touch. In operation, the cotton squares are rubbed over the revolving rollers which seize the leaf bracts and tear them from the squares.

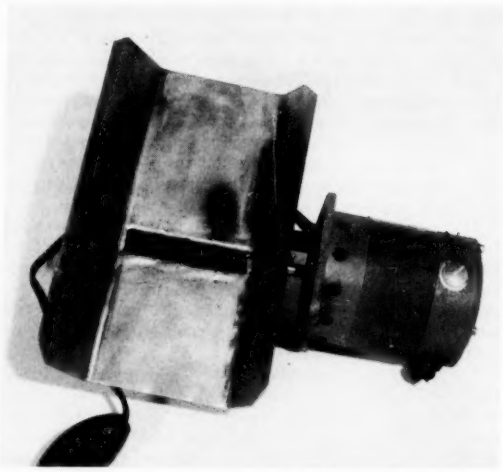

FIG. 1.—A motor-driven set of rollers.

The rollers should be as small in diameter as possible to avoid catching the square itself. The motor may be mounted directly to the roller frame with the end of one roller fixed to the motor shaft, or the end of the roller may simply be held in the chuck of a small electric drill. The roller speed should be rather rapid (3,000 to 5,000 r.p.m.) for maximum efficiency. Slower speeds will work but the rollers tend to catch the squares.

Table 2.—Per cent of boll weevil eggs recovered from squares by two methods of extraction.

Test	Number of Eggs: Blender-Salt Extracted	Number of Eggs: Hand Extracted	Per Cent
1. a	109	185	59
b	121	178	68
2. a	45	105	43
b	60	128	47
3. a	110	157	70
b	128	150	85
		Average	62

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Effectiveness of Lindane, Malathion, Methoxychlor, and Pyrethrins-Piperonyl Butoxide against the Hairy Spider Beetle, *Ptinus villiger*[1]

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The hairy spider beetle, *Ptinus villiger* (Reit.), is the most prevalent pest of commercial flour storages in Western Canada. It infests flour and feeds when the female lays eggs through the mesh of cotton and jute sacks. Oviposition begins in April when adults invade unheated warehouses from overwintering sites beneath the floor or from sacked animal feeds stored inside. Oviposition rate is slow during cool spring weather, increases rapidly with the onset of summer, and declines in July. Thus, adequate protection must be provided against adult beetles, especially during peak oviposition.

In food storage warehouses residual insecticides are applied to the floors and walls to kill insects before they reach sacked cereal products. Careless application of such insecticides involves the risk of contaminating foodstuffs with dangerous residues. Thus it is desirable to recommend insecticides of low mammalian toxicities for use near stored foodstuffs.

Smallman (1948) reported that a single application of 5% DDT at 4.1 litres per 1,000 sq. ft., in April, gave good control of spider beetles during the egg-laying period. Evaluation of 2.5% DDT, 2.5% methoxychlor and 0.25% pyrethrins-2.5% piperonyl butoxide applied at 0.9 gal. per 1,000 sq. ft. (Watters & Smallman 1953) showed that two applications of pyrethrins-piperonyl butoxide, in April and June, were needed to give control equivalent to one application of DDT in April. Methoxychlor was considerably less effective than DDT or pyrethrins-piperonyl butoxide.

DDT is considered safe to use near foodstuffs provided it doesn't contaminate the commodity. Methoxychlor, malathion and pyrethrins-piperonyl butoxide, which have a lower acute mammalian toxicity than DDT (Handbook of Toxicology 1959), are sometimes preferred because of the greater margin of safety. Lindane, although having a higher acute oral toxicity than DDT to mammals, is faster acting and more toxic to many stored product insects (Parkin 1955) and, consequently, may be applied at lower concentrations than DDT to obtain comparable control.

This is a report of experiments carried out in warehouses to compare the residual effectiveness of lindane, malathion, methoxychlor and pyrethrins-piperonyl butoxide against *P. villiger*.

MATERIALS AND METHODS.—The four insecticides were assigned on a random basis to 32 flour and feed storage warehouses in Manitoba. Since all warehouses were being used for commercial storage of flour none were left untreated as controls. The following insecticides were applied to the floors and walls of warehouses at 1 gal. per 1,000 sq. ft.: 1% lindane, supplied as 50% wettable powder; 5% methoxychlor, supplied as 50% wettable powder; 0.1% pyrethrins-1.0% piperonyl butoxide, supplied as 5% pyrethrins-50% piperonyl butoxide and diluted with deodorized base oil; 3% malathion, supplied as 50% emulsion.

Eight 1-pound cotton sacks of flour were distributed on the floor of each warehouse after treatment. The insecticide treatments were assessed by the number of spider beetle progeny sifted from the flour. The sacks were replaced with fresh sacks of flour at 4, 6, 8 and 12 weeks after treatment and sifted to determine when the insecticides became ineffective. Three of the warehouses treated with malathion were retreated after 6 weeks when high insect counts were noted at the first assessment. All other warehouses were retreated after 8 weeks.

RESULTS.—Figure 1 shows the comparative protection afforded sacks placed in warehouses treated with the four insecticides. There were no significant differences among the insecticides at the first assessment, 4 weeks after application. But between 4 and 6 weeks, malathion failed to give adequate control of spider beetles when compared with the other treatments ($P<0.01$). Pyrethrins-piperonyl butoxide, also, gave less protection to flour stocks at 6 weeks than at 4 weeks after application ($P<0.01$), as did methoxychlor ($P<0.05$). There was no significant difference between the 4- and 6-week assessments in warehouses treated with lindane but there was a difference ($P<0.05$) between the 4- and 8-week assessments.

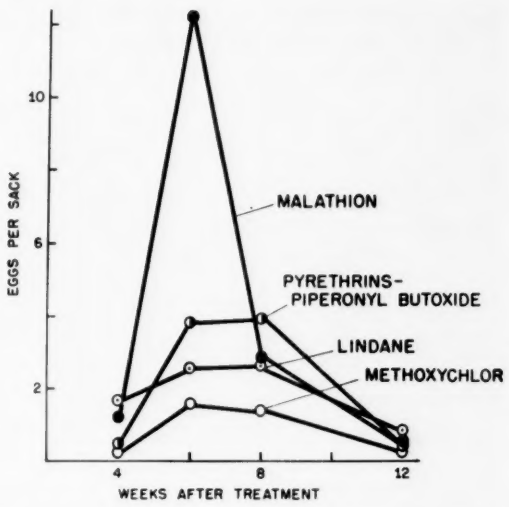

FIG. 1.—Numbers of eggs of *Ptinus villiger* laid in flour sacks in warehouses after treatment on April 15 and retreatment after 8 weeks.

The large drop in oviposition ($P<0.01$) at 8 weeks, in warehouses treated with malathion, occurred because three of the warehouses showing the heaviest counts at the 4- and 6-week assessments were retreated after 6 weeks. A second application of all treatments, after 8 weeks, apparently gave improved control, but the low egg counts in test sacks at 12 weeks may also be attributed to a seasonal decline in oviposition rate.

Throughout the summer, methoxychlor was superior to malathion and pyrethrins-piperonyl butoxide ($P<0.05$). A multiple range test (Duncan 1955) showed that there was no significant difference between methoxychlor and lindane, nor between lindane and pyrethrins-piperonyl butoxide.

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Endrin Residues on Corn Stover in Relation to Dairy Cattle Feeding[1]

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Endrin is one of several insecticides currently recommended by the U. S. Department of Agriculture (Anonymous 1960) for the control of the European corn borer (*Pyrausta nubilalis* (Hbn.)). Farmers in the Corn Belt frequently pasture their cattle in corn fields after harvest. The cattle pick up ears missed in harvesting and also feed on the stover. The objective of this experiment was to determine if any endrin would be secreted in the milk of dairy cattle allowed to pasture on corn stover remaining in the field after harvest of the endrin-treated corn.

A 4.4-acre field of dent corn was treated with a granulated formulation containing 2.5% endrin at the rate of 0.25 pound of endrin per acre. An adjacent 4.7-acre field was used as the untreated control. The insecticide was applied for the control of first-brood corn borers when the corn was in the late whorl stage. An experimental fluted-feed granular applicator, mounted on a high-clearance vehicle, was used to apply this treatment.

After the corn was picked in the fall, five lactating dairy cows were randomly assigned to pasture in the fields; two in the untreated field and three in the endrin-treated field. Each field was enclosed with electrified wire. Milk samples were collected from each group of cows twice weekly over a period of 38 consecutive days. The cream was separated, chilled overnight, and made into butter. The butter was washed and stored in polyethylene bags at −15° C. Additional details of the insecticide application, pasturing and care of the dairy cows, milk processing, and butterfat preparation are the same as described for an earlier study of heptachlor residues by Johnsen *et al.* (1960).

The quantitative estimation of endrin residues in butterfat was based on methods developed by Bann *et al.* (1958) supplemented by the following modifications. The saponified butter extract was chromatographed through a column approximately 18 mm. in inside diameter, containing the following materials from bottom to top: a glass wool plug, about 1 cm. anhydrous sodium sulfate, 10 cm. (14.2 gm.) 60/100 mesh Florisil[3] conditioned to contain 8% water, 2 cm. (1.65 gm.) 1:2 (w/w) magnesia(MgO)-Celite mixture, 2 cm. (1.3 gm.) Norit-A decolorizing carbon, and about 3 cm. anhydrous sodium sulfate. The column was wetted with purified Skellysolve B, after which the saponified extract, reduced in volume to about 10 ml., was poured quantitatively onto the column and washed into the column with a few milliliters of Skellysolve B. The column was eluted with 150 ml. of purified Skellysolve B, the eluate discarded, and the column eluted with 200 ml. of 15% (v/v) diethyl ether in colorimetric grade pentane. The eluate contained the endrin.

The color developing reagent was modified as follows according to suggestions of Fahey & Schechter (1960): (1) 0.25% sulfanilic acid, prepared by dissolving at 50° C., 0.25 gm. of sulfanilic acid in 100 ml. of a distilled water solution containing 50% glacial acetic acid by volume, (2) 0.1% aqueous solution of sodium nitrite, and (3) 0.5% aqueous solution of ammonium sulfamate. Five volumes of sodium nitrite solution were mixed well with 4 volumes of sulfanilic acid solution and allowed to stand 5 minutes. One volume of ammonium sulfamate solution was then added and mixed well before use.

Other modifications of the analytical procedure included: (1) The hot dechlorination mixture was cooled to room temperature by immersion in cold water. The condenser was rinsed with 10 ml. pentane followed by 10 ml. distilled water. This cooled solution was transferred to a 500 ml. separatory funnel containing 50 ml. pentane and 200 ml. distilled water; (2) The pentane extract from the preceding procedure was reduced in volume using a 3-ball Snyder column, keeping the water bath temperature below 50° C.

Eleven composite milk samples were collected from both groups of cows during the 38 days they were pastured on the two fields. Fifty-gram butterfat samples were used for the following analyses: (1) 11 samples from the control cows, (2) 11 samples from cows on the endrin-treated plot, and (3) 11 samples of control butterfat to which had been added 2 p.p.m. endrin. Each time a set of these three samples was analyzed, a 100-μg. sample of endrin[4] was also carried through the analytical procedure. A reagent blank was also prepared each time. Several standard curves were also prepared; the endrin quantities usually used for this purpose were 30, 50, and 70 μg. An average standard curve based on these quantities had a slope of 0.017 absorbance units per microgram of endrin. All samples were read on a Beckman model DU spectrophotometer at 515 mμ. in cuvettes with a 1-cm. light path.

The absorbance values of the 11 butterfat samples analyzed for endrin were never greater than the values for the corresponding control samples. The reagent blanks carried through the procedure were water white and had an average absorbance value of 0.000. The recovery of 2 p.p.m. of endrin added to 50-gram control butterfat samples averaged 61%. The modified color developing reagent did not develop color on standing for as long as 24 hours and was very stable when acidified with 50% sulfuric acid.

Great care must be exercised during chromatographic separation of the endrin, reduction in volume of the dechlorinated extract, reaction of the dechlorination mixture with phenyl azide, and removal of excess phenyl azide under vacuum. The optimum conditions for removing the excess phenyl azide in these experiments were 225 mμ. vacuum for 5 minutes in a water bath of 60±1° C. The modifications which Fahey & Schechter (1960) have developed for the Bann *et al.* (1958) procedure have eliminated the variable background color which appeared in the reagent blanks due apparently to the coupling of excess sulfanilic acid with the diazotized sulfanilic acid. It is noteworthy that other reports of endrin analysis using the Bann *et al.* (1958) procedure (Kiigemagi *et al.* 1958, Terriere *et al.* 1958, 1959) make no mention of these difficulties.

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[1] Journal Paper No. J-4009 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa. Projects No. 1336, 1351, and 1435. This study was supported by a research grant RG-4066, from the National Institutes of Health, Division of Research Grants, Public Health Service and is also a contribution from North Central Regional Project NC-33. Accepted for publication November 18, 1960.

[2] The first two authors are from the Department of Zoology & Entomology and the fourth author from the Department of Animal Husbandry, Iowa State University of Science and Technology. The third author was associated with the Grain and Forage Insects Research Branch (Ankeny, Iowa), Entomology Research Div., U. S. Department of Agriculture at the time these studies were begun. He is currently a member of the Department of Entomology, Univ. of Missouri, Columbia.

[3] The Florisil was supplied by the Shell Development Co., Modesto, Calif.

[4] Recrystallized endrin (98.2% purity) was supplied by the Shell Chemical Co., New York 20, N. Y.

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An Insecticide Vaporizer for Treating Small Enclosed Areas[1]

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The vapors from the organophosphorus insecticides have been known for several years to be toxic to insects. This toxicity was first observed in treated greenhouses when they were closed during cold weather or at night (Smith *et al.* 1948).

Recently there has been some interest in the use of the less toxic organophosphorus compounds for space control of insects. In most applications the insecticides have been used as sprays, aerosols, or by painting the floors, walls, or other surfaces of warehouses, greenhouses, or similar buildings. Where no circulation is provided, these methods, other than by aerosol, require many hours to reach a concentration of insecticide in the air that is lethal to insects. The aerosol method requires that the operator wear elaborate protective clothing until the particles settle or evaporate.

In search of a simpler method of vaporizing formulations containing the safer organophosphorus insecticides, we carried out tests with a wick wet with one of them and exposed in a small room. A solution containing 2 grams of DDVP in 12 grams of xylene was placed in a 20-ml. test tube. A cotton wick, three-eighths inch in diameter by 3 inches long, was put into the open end of the test tube through a cork stopper. The tube was then placed on its side on an aluminum plate on the floor. In this way the exposed wick remained saturated until the entire contents of the test tube had evaporated.

Two of these test tubes were used in a 1,000-cubic-foot room, with caged adult Japanese beetles (*Popillia japonica* New.) as the test insects. After a 2-hour exposure to the vapor at 25° C., the beetles were removed and held in untreated air. After a 24-hour holding period 47% of the beetles were dead, and 30% were moribund. A 16-hour exposure to the vapor produced a mortality of 99%, with 1% moribund, in 24 hours. Approximately 0.75 gram of the solution evaporated per hour from each wick.

In order to speed up the evaporation rate, another test with Japanese beetles was run in which a small battery-operated vacuum cleaner[2] was used to blow air over the wick (fig. 1). One gram of DDVP in 6 grams of xylene was placed in a 10-ml. test tube with the same sized wick as in the previous test. The exposed end of the wick was inserted through a hole cut in the vacuum cleaner bag. Figure 1 shows this wick exposed. The vacuum cleaner was placed on its side since the wick remained saturated in this position. The cleaner was then turned on. Only one unit was used in the 1000-cubic-foot room.

An exposure of 1 hour at 23° C. produced a 70% mortality of the beetles, with 30% moribund, after a holding period of 24 hours; 100% were dead in 4 days. The evaporation rate from the wick was approximately 3.5 grams per hour.

FIG. 1.—A battery-operated vacuum cleaner with a test tube containing a wick attached. The wick is put through a hole in the bag when ready for use.

Since these small vacuum cleaners are inexpensive and easy to operate and the batteries last for many hours, they are practical for treating small areas. An alternative for the test tube and wick would be a throw-away bag containing an ampule of DDVP formulation imbedded in cotton. When treatment is necessary, the ampule could be broken and the apparatus operated until the solution evaporates. When time is not a factor, the ampule could be broken and the bag exposed without the use of the vacuum cleaner. Dibrom[3] (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate) or similar organophosphorus compounds could be substituted for DDVP. The ampules in the throw-away bags could also be used in regular electric-type vacuum cleaners.

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[1] Accepted for publication November 18, 1960.

[2] This type of device, which is commonly sold in department stores and haberdasheries for about $1.00 under the name of battery-operated vacuum cleaner, combines the functions of a small vacuum cleaner and a flashlight.

OBITUARY

Walter M. Kulash
1912-1960

Dr. Walter M. Kulash, Research Professor of Entomology, North Carolina State College, Raleigh, died after a brief illness at Raleigh, North Carolina, on September 18, 1960, at the age of 48. Dr. Kulash was born at Haydenville, Massachusetts, January 8, 1912. He attended the University of Massachusetts where he received his B.S., M.S., and Ph.D., the last having been awarded in 1942. In 1942 Dr. Kulash joined the staff at North Carolina State College where he was very active in teaching and in applied entomology, especially in connection with wireworms and insect pests that attack tobacco, corn, cotton, and other farm crops.

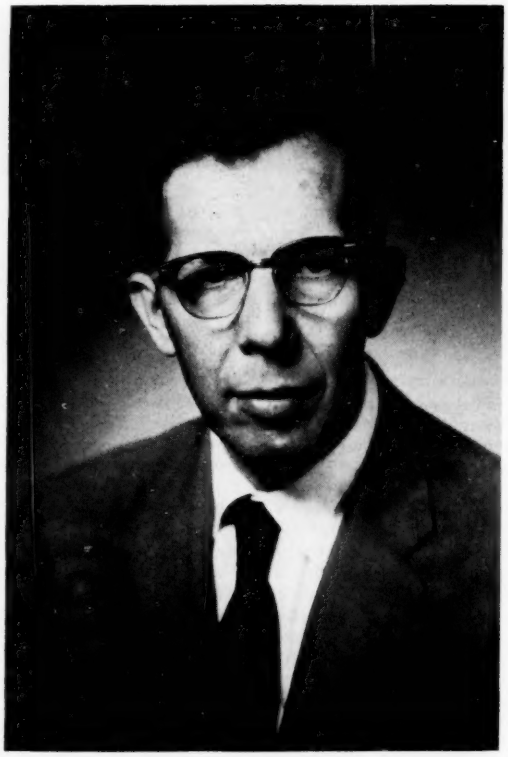
Dr. Kulash was a member of many professional and scientific societies, among them the Entomological Society of America, Sigma Xi, the Society of Systematic Zoology, the Coleopterists Society, the American Association of University Professors, and the North Carolina Entomological Society.

Dr. Kulash is survived by his widow, Mrs. Mary Gobush Kulash; a daughter, Sister Mary Stephanie, of Mount St. Mary's Novitiate, in Newburg, New York; five sons, Walter M., Jr., John A., Dameon J., Joseph A., and Francis M., all of the home.

Dr. Kulash was a former president of the North Carolina Entomological Society, former president of the State College Chapter of the American Association of University Professors, former secretary of the Zoology Section of the North Carolina Academy of Science and a former president of the North Carolina Catholic Laymen's Association.

Dr. Kulash had a rich and many-sided life. He was a student of philosophy and languages. He was deeply interested in the development of the students, the department, the college, and the community. He was author or co-author of more than 67 technical articles, publications, and scientific manuals. He exemplified selfless dedication to his church, to his family, and to his work.

CLYDE F. SMITH



Walter M. Kulash
1912–1960

Treatment of Peach Nursery Stock to Prevent Infestation by the Peach Tree Borer[1]

OLIVER I. SNAPP, *Entomology Research Division, Agric. Res. Serv., U.S.D.A.*[2]

Methods of treatment are needed to protect peach nursery stock from damage by the peach tree borer (*Sanninoidea exitiosa* (Say)), since borer-injured stock is unsalable. Tests of trunk sprays for borer control were made in a small peach nursery in the 1956–57 and 1957–58 production seasons. The treatments were applied during the growing season with a hand sprayer in 1956 and a power sprayer in 1957. The degree of borer infestation and extent of damage to the trees were determined during the spring following treatment while the stock was being dug.

Results of the treatment of 816 trees in the 1956–57 tests and 1,045 in the 1957–58 tests are given in table 1. The 2-year-old trees that received the spray treatments in 1957 were protected from borer attack in 1956 by application of DDT, as reported in the table for the 1956–57 season. DDT used in four applications gave complete control, dieldrin in a single application good control, and parathion in three applications fair control.

Table 1.—Peach nursery trees infested with the peach tree borer after treatment with sprays, Fort Valley, Ga., 1956–58.

TREATMENT (LB. WETTABLE POWDER PER 100 GAL.)	DATES OF APPLICATIONS	INFESTED TREES AT INDICATED AGE (%)	
		1 Year	2 Years
	1956 Season		
DDT (50%), 8	July 12, Aug. 9, Sept. 14, Oct. 9	0.0	—
Untreated check	—	11.5	—
	1957 Season		
Dieldrin (50%), 6	Aug. 1	0.8	2.4[a]
Parathion (15%), 2	Aug. 1, Sept. 3, Oct. 7	9.4	3.6[a]
Untreated check	—	30.1	41.7

[a] Treated with DDT as above in 1956 season.

[1] Accepted for publication January 12, 1961.

[2] Julius P. Hollon assisted with the experiments reported in this paper.

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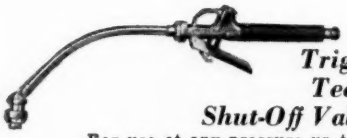

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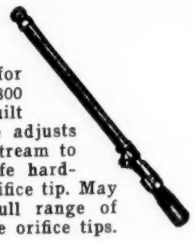

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